Microhabitat and elevational patterns in thermal tolerance and thermoregulation of Neotropical army ants (Formicidae: Dorylinae)

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by

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Dedication

This work is dedicated to the tiny lives that affect us all and to the tremendous individuals who teach us to notice them.

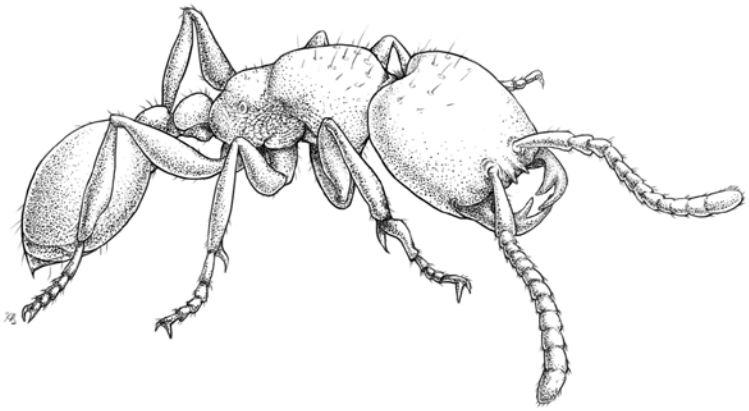

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Table of Contents

List of Tables

List of Figures

Abstract

Microhabitat and elevational patterns in thermal tolerance and thermoregulation of Neotropical army ants (Formicidae: Dorylinae)
Kaitlin Mari Baudier
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Temperature is a major abiotic factor governing organismal performance and biotic distribution patterns worldwide. How different spatial resolutions of temperature variation relate to the thermal adaptiveness of organisms living within these environments informs predictions of physiological range limitations and response to climate change. Here I use Neotropical army ants as a model system to test broadly developed macrophysiological predictions on the scale of tropical microclimates and elevation clines. Social insect colonies make particularly interesting models for this purpose, as they enable the testing of predictions on both the level of the individual and the social group (colony). Here I form a new set of predictions for how insect societies are constrained and benefited by their thermal environments. I demonstrate novel patterns in tropical thermal tolerance due to soil microhabitat, elevation, and seasonality and discuss how these patterns interplay with the adaptiveness of castes in ant colonies. I also report novel differences in social thermoregulation across an elevational thermal cline and across brood ages in an above-ground nesting army ant species with a detailed study of thermal tolerance and thermoregulation in a below-ground army nest. The findings of this work suggest benefits in the incorporation of detailed microclimate use and social parameters in models predicting the ecological effects of climate change. These projects also form new testable hypotheses for future work relating to the thermal ecology of social insects.

CHAPTER 1: WEAK LINKS AND TEMPERATURE EXPLORERS: HOW INDIVIDUAL DIFFERENCES IN THERMAL PHYSIOLOGY CONSTRAIN AND ENABLE ANIMAL SOCIETIES

An invited review article (Baudier & O'Donnell *In revision*)

Introduction

Temperature variation is a major selective force on the thermal physiology of biota worldwide, driving diversification across latitudes, elevations, and microhabitats (Janzen 1967; Ghalambor *et al.* 2006; Kaspari *et al.* 2015). I suggest that negative fitness effects of both heat and cold stress can be amplified in social species, using social insects as models. An animal's thermal tolerance range is the breadth of temperatures at which it can function; the upper functional limit is the critical thermal maximum (CT_{max}), and the lower limit is the critical thermal minimum (CT_{min}) (Angilletta 2009). Thermal tolerance metrics are frequently used to estimate fitness costs of climate variation and climate change

with particular emphasis on small-bodied ectotherms with body temperatures near ambient (Pörtner 2001; Deutsch *et al.* 2008; Sunday, Bates & Dulvy 2011; Huey *et al.* 2012; Buckley & Huey 2016). Thermal tolerance metrics have been applied to assess the potential impacts of climate change on social insect populations and communities (Diamond *et al.* 2012; Oberg, Toro & Pelini 2012; Kingsolver, Diamond & Buckley 2013; Warren & Chick 2013; Bishop *et al.* 2016). However, these analyses have not accounted for the effects of individual thermal tolerance differences within colonies.

Individual workers can differ significantly in thermal tolerance within social insect colonies (Mitchell, Hewitt & Van Der Linde 1993; Cerda & Retana 1997;

Clemencet *et al.* 2010; Ribeiro, Camacho & Navas 2012; Oyen, Giri & Dillon 2016), and these differences may confer either benefits or costs to the colony. **I. Social benefits:** If a species evolves a new higher-tolerance worker class that allows exploitation of new thermal environments, then worker thermal diversity can be an advantage (Cerdá 2001). Colonies can also respond to temperature in ways individual members cannot. Though most individual social insects are poikilotherms there can be emergent thermal physiology at the colony level, such as nest thermoregulation. Workers that differ in thermal sensitivity can stabilize colony temperature homeostasis (Jones *et al.* 2004). **II. Social costs:** If workers encountering a thermally challenging environment differ in thermal tolerance, then colonies will likely incur performance costs due to negative effects on the most thermally sensitive individuals. I term this the "weak link hypothesis." I propose that models predicting species distributions and effects of climate change on social insect populations may underestimate risks to social species if thermal weak-link costs are not taken into account.

Behavioral predictions

Here I explore three alternative predictions of social insect behavioral responses to challenging thermal conditions, given weak-link pressures, noting the putative group-level costs and benefits of each. Which response colonies make affects how social insect populations can respond to temperature variation.

1. Mortality aversion: Groups avoid exposure to temperatures that would stress the most sensitive workers (Figure 1). If observed, this would suggest some individuals'

behavioral responses to temperature are mismatched from their own physiological aptitude. The colony benefit would be protection of all individuals from physical harm. The cost would be not exploiting some resources in thermal environments that are accessible to the most tolerant individuals.

2. Colony gain maximization: Groups are relatively insensitive to elevated temperatures, exposing weak links to harmful thermal environments (Figure 1). Colony level costs of this strategy include higher risk of mortality or thermal stress of weak links. However, colonies benefit from the full worker force exploitation of resources in extreme thermal environments. Foragers of several army ant species have been observed raiding over short distances at temperatures which exceed the CT_{max} of small castes (Baudier *et al.* 2015), suggesting that this response occurs in nature.

3. Self preservation: Individuals respond to environmental temperature in a manner appropriate to their own thermal tolerance (Figure 1); weak links avoid working in temperatures stressful to their own physiologies. This could be costly to the group by reducing efficiency if all size classes contribute useful labor to the tasks performed in the thermally challenging environment. An example of this strategy is the evolution of especially thermally tolerant castes in desert *Cataglyphis* which exploit high-temperature resources independently of other workers, often foraging at different times of day than sensitive nestmates (Cerda & Retana 1997; Cerdá 2001). Social insect colonies also typically house sensitive colony members such as juveniles or recently molted individuals in different parts of the nest that experience less thermal variation, creating for them a self-preservation environment to wait out their sensitive developmental stages

while sometimes performing specific tasks within the nest (Jones & Oldroyd 2006; Baudier & O'Donnell 2016).

Causes of critical temperature diversity

Multiple factors can cause within-colony variation in thermal tolerance. Size-polymorphic worker castes are present in about 10% of ant species, including major ecological players like fire, army, and leaf cutter ants, as well as in termites (Wilson 1968; Hölldobler & Wilson 1990). Within polymorphic social insect colonies larger workers have higher CT_{max} (Ribeiro, Camacho & Navas 2012; Oyen, Giri & Dillon 2016; Wendt & Verble-Pearson 2016), lower CT_{min} (Clarke, Thompson & Sinclair 2013), and wider thermal tolerance breadths (Kaspari *et al.* 2015). Large-bodied castes also have greater thermal inertia, causing slower heating and cooling and more resistance to short duration heat stress than smaller castes (Kaspari *et al.* 2015).

Within monomorphic and polymorphic species alike, genetic and epigenetic variation among colony members can cause diverse individual responses to temperature stress. Differences in heat sensing among genetic patrilines in polyandrous *Apis melifera* colonies cause differences in thermoregulatory responses among nestmates (Jones *et al.* 2004). Differences in gene expression between acclimated and non-acclimated nest mates can also affect worker responses to thermal stress (Cahan *et al.* 2017). Up-regulation of heat shock proteins in the Small Hsp, Hsp40, Hsp60, Hsp70, and Hsp90 families is linked to improved thermal performance among acclimated eusocial hymenoptera (Gehring & Wehner 1995; Nguyen, Gotelli & Cahan 2016). Heat shock protein synthesis can also be

induced by cold stress, aiding cellular function in both directions of extreme temperature exposure (Burton *et al.* 1988). If only some workers are exposed to extreme temperatures while foraging, they may have advantages in near future extreme temperature scenarios over nestmates. Sequence divergence among workers at thermally responsive loci, or changes to their expression patterns, can create within-colony heterogeneity in worker thermal tolerance.

Worker age is another important determinant of thermal tolerance among nestmates. Juvenile, young adult, and senescing individuals have different thermal tolerance ranges and acclimation response across many insect species (Bowler & Terblanche 2008). In the harvester termite *Hodotermes mossambicus,* increasing sclerotization of the cuticle over time is associated with increased thermal tolerance breadth among mature workers as compared to un-pigmented larvae (Mitchell, Hewitt & Van Der Linde 1993). Similarly, workers of the army ant species *Labidus praedator* have a higher CT_{min} immediately following eclosion from the pupal stage as compared to after several days of cuticle hardening (Baudier & O'Donnell 2016).

Dynamics of environmental variability

Over an evolutionary time scale, I predict that changes in the magnitude of extreme ambient temperatures can result in changes to selection pressure against weak links (Figure 2). There are two key issues that affect a social insect species' persistence in a thermal environment: thermal tolerance breadth (temperatures between mean CT_{max} and mean CT_{min}) and thermal tolerance diversity (variation among workers in CT_{max} or CT_{min}).

While many studies have focused on thermal tolerance breadths in predicting species thermal sensitivity (Navas 1996; Gaston & Chown 1999; Sunday, Bates & Dulvy 2011), thermal tolerance diversity is poorly understood. I suggest this diversity has high relevance for social species, where interactions among individuals with different thermal sensitivities can affect group performance.

I can also consider the potential social benefits of individuals with high CT_{max} and low CT_{min}, or "strong links." Environmental temperature challenges are less likely to affect these individuals compared to weak links, but colony-level net benefits of producing strong-link individuals depend on both physiological and abiotic factors. In some cases strong-link castes evolve due to selection pressure on resource access in thermally extreme environments (Cerda & Retana 1997; Cerdá 2001). However, strong links can also evolve as a byproduct of other selection pressures. Large-bodied majors of *Solenopsis geminata* are thought to have evolved muscular seed-crushing heads coinciding with a dietary shift towards granivory (Wilson 1978). Similarly, the dietary shift from collecting frass to leaf-cutting in higher attines coincides with an increase in worker polymorphism, including the addition of a large worker caste to carry leaves and even larger soldiers providing colony defense (Hölldobler & Wilson 1990). Increased worker body size in *Solenopsis* and *Atta* is also associated with higher CT_{max} (Ribeiro, Camacho & Navas 2012; Wendt & Verble-Pearson 2016), causing potentially incidental strong links. However, there are costs associated with producing strong links. Colony production of large-caste individuals requires more nutrient provisioning (Calabi & Porter 1989). Heat or cold acclimation may increase short-term survival in extreme

temperatures, but it also can decrease long-term survival and reduce efficiency of task performance (Scott, Berrigan & Hoffmann 1997; Kristensen *et al.* 2008).

If in a given species' strong links and weak links are both of net cost to the colony (for potentially different reasons), then low thermal tolerance diversity is predicted as a result of stabilizing selection (Figure 2). If the sum colony-level benefits of both strong links and weak links outweigh the costs, then high thermal tolerance diversity is predicted. However, if colony-level costs and benefits are equal for strong links and weak links, then thermal tolerance diversity is predicted to be dependent on physiological limits due to phylogenetic constraints. There is evidence for high weak-link costs and possible net gain from strong links among surface-active Neotropical army ants (Baudier *et al.* 2015). In this study obligate below ground species of army ants encountered little diel variation in temperature and solar input relative to surface-active species. Above-ground species, which evolved from a subterranean ancestor (Brady *et al.* 2014; Borowiec 2016), not only had wider mean thermal tolerance breadths, but also had lower thermal tolerance diversity than below-ground species. Body size was positively related to CT_{max} in all species sampled, but the size-CT slope was steeper in below-ground species. This partial decoupling of body size and thermal tolerance suggests factors other than size act to set thermal tolerance diversity, but that variable size also likely imposes an inherent weak-link effect.

Latitudinal gradients: linking climate and caste diversity

Latitude is likely the best-studied ecological cline in thermal variation. Globally, ants follow Bergman's rule of increasing body size towards high latitudes both in terms of

individual worker size (Cushman, Lawton & Manly 1993) as well as colony size (Kaspari & Vargo 1995). According to the size–complexity hypothesis, worker size polymorphism among social insects is more likely to evolve in larger colonies (Bonner & Brainerd 2004; Ferguson-Gow *et al.* 2014), which would lead to the prediction that worker physical polymorphism in ants would be more common at high latitudes. However ant colonies are more size-polymorphic in the tropics (Wilson 1968). I suggest that increasing worker size polymorphism may be more likely to evolve in the tropics where environmental temperature variation pressures against weak-link castes (i.e., small bodied workers) are relieved. That ants tend to be larger in individual size (Cushman, Lawton & Manly 1993) as well as more uniform in size (Wilson 1968) in the temperate zone suggests a possible loss of small heat-sensitive and cold-sensitive castes as more variable seasonal temperatures drive mean worker size to be greater and less diverse.

Climate change

Rapid increase in both global mean temperature and climatic variability are major predictions of anthropogenic climate change (Hartmann, Tank & Rusticucci 2013; Strauss, Formayer & Schmid 2013). Under these changing conditions weak-link individuals are the first colony members to incur physiological costs due to climate change. These costs inherently affect overall colony fitness. Due to the weak-link effect, strong climate change costs to social species can occur before the mean critical thermal limits of a species are exceeded. These social costs are poorly assessed by current models,

as standard practice is to use species means and not colony outliers to predict the impacts of directional increase and variation increase on social species.

Due to little seasonal variability in temperature, tropical species often have narrow mean thermal tolerance breaths, making them more vulnerable than high latitude species to small changes in temperature (Huey *et al.* 2009; Sunday, Bates & Dulvy 2011). However, tropical ant species have the added challenge of potentially magnified weak-link effects due to higher incidence of small, thermally sensitive castes.

Complications and qualifying factors

If the weak-link effect is so costly for colonies, why are extra-sensitive colony members present, even in low-variability environments? These patterns suggest that there is some cost to the evolution of a uniformly widely tolerant colony. In relatively benign thermal environments, the benefits of worker heterogeneity may outweigh weak-link costs. Heterogeneity in thermal sensitivity contributes to more stable thermoregulation in nests of *Apis melifera* (Jones *et al.* 2004). In large colonies task specialization also drives polymorphism (Ferguson-Gow *et al.* 2014), causing thermal tolerance diversity independent of thermal adaptiveness.

Lastly, although most empirical studies suggest a positive relationship between colony member body size and CT_{max} and a negative relationship between body size and CT_{min}, the exact mechanism of this relationship is the result of a wide variety of factors, some of which are understudied. This is evidenced by results of between-species body

size relationships that contradict within-species predictions of thermal tolerance increase with body size (Oberg, Toro & Pelini 2012; Verble-Pearson, Gifford & Yanoviak 2015).

Implications for future research

An important implication of the weak link hypothesis is a reevaluation of how thermal tolerance metrics predict climate change effects in social species. Most studies investigating vulnerability of social insects to climate change base risk assessments on species averages, disregarding negative effects on thermally sensitive individuals (Käfer, Kovac & Stabentheiner 2012; Oberg, Toro & Pelini 2012; Ribeiro, Camacho & Navas 2012; Bishop *et al.* 2016). Models of social insect species' vulnerability to thermal stress should include metrics of colony minimum tolerance and colony tolerance diversity. Incorporating these colony-level effects in performance assessments will increase accuracy of predictions within and among social taxa.

Empirical studies testing the three behavioral predictions of weak-link pressures are also needed. Whether social species engage in *mortality aversion*, *self preservation*, or *colony gain maximization* relates to tradeoffs in altruism and task specialization. The weak link hypothesis adds to this already complex discussion of biotic interactions, introducing a new level of abiotic complexity. A broad survey of weak-link effects across social insect taxa can be conducted via experiments that alter the thermal environment of worker tasks, observing behavioral response followed by thermal tolerance assays.

Assessment of geographic and microhabitat patterns in colony size-polymorphism and thermal tolerance diversity are paramount in testing if and how weak-link factors

play into large scale patterns of thermal physiology. Such work would shed light on the interaction between colony size, task specialization, cast differentiation, and abiotic factors.

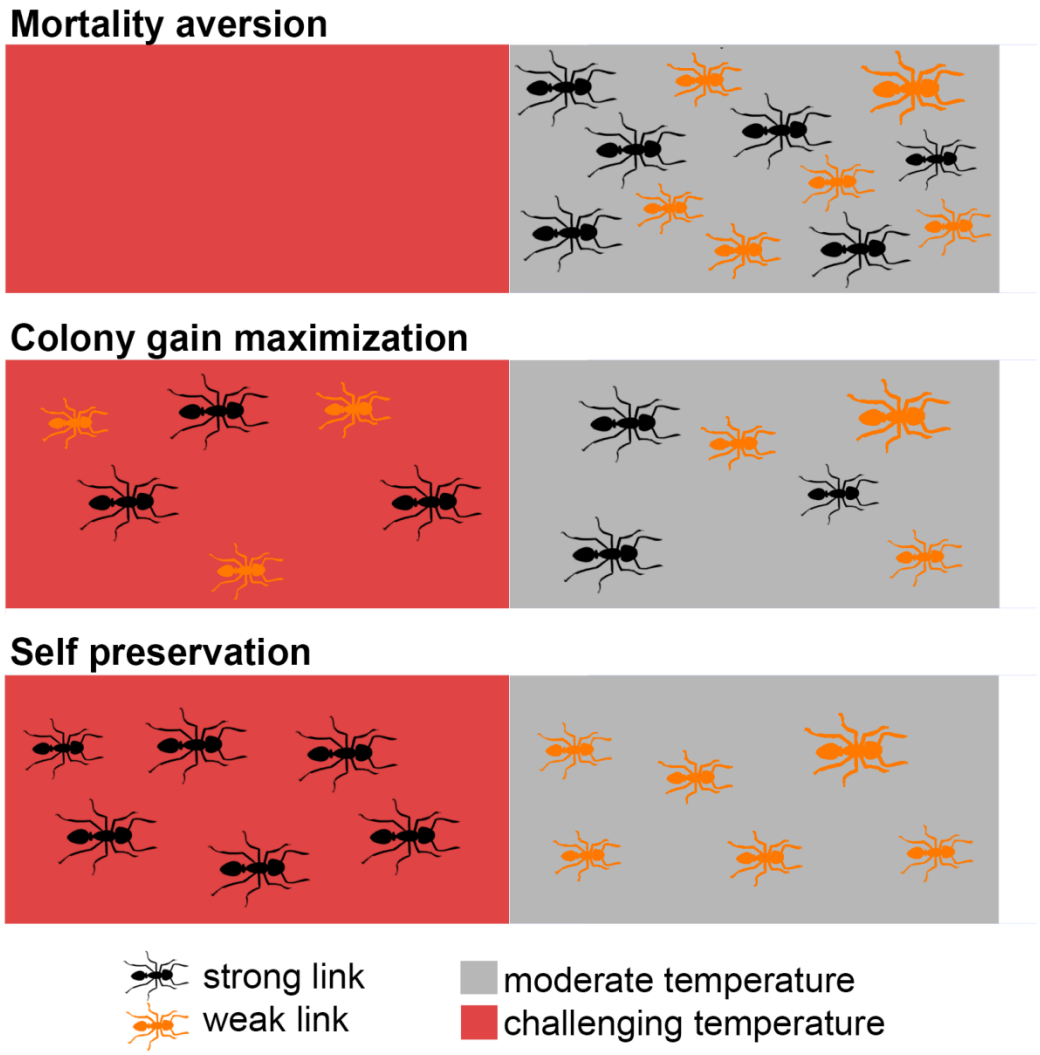

Figure 1-1. Predicted alternative behavioral response strategies to weak-link effects: Mortality aversion, Colony gain maximization, and Self preservation; within a colony orange nestmates ("weak links") are more sensitive to extreme temperature than strong link nestmates (depicted in black).

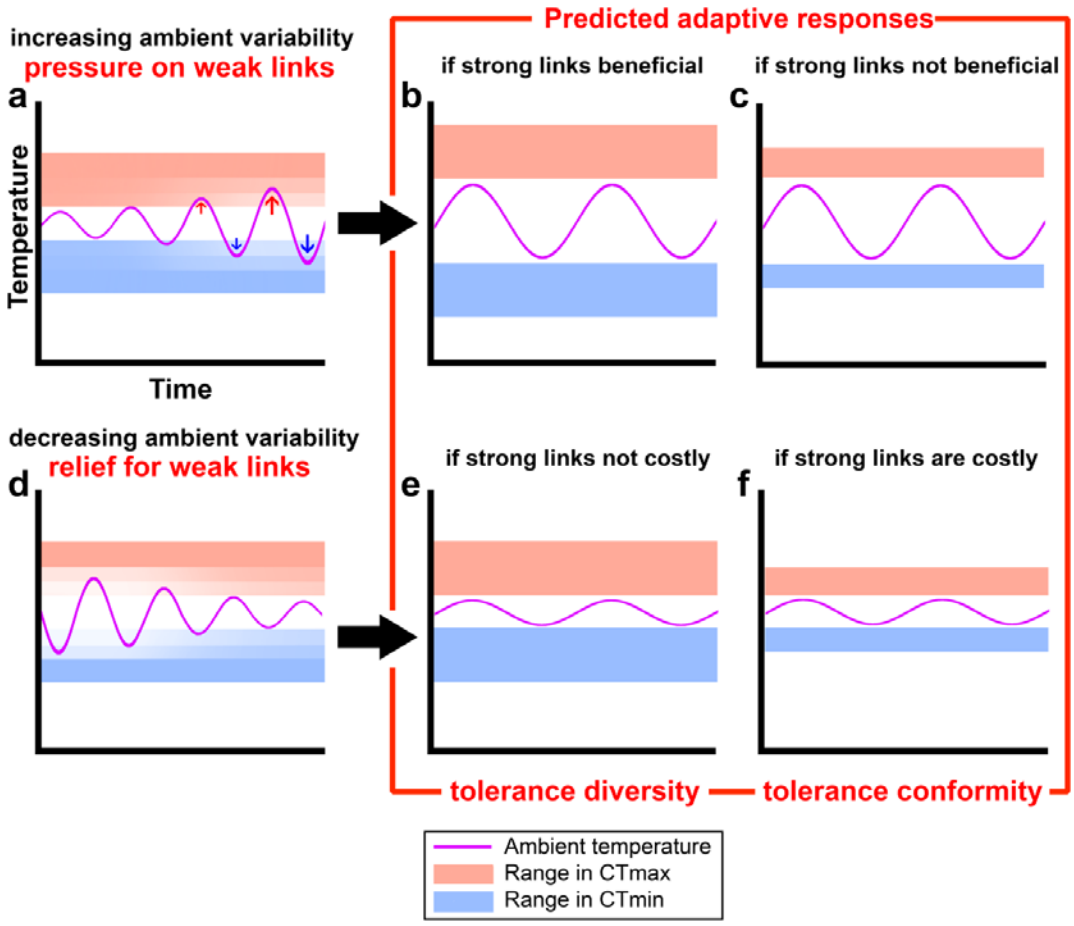

Figure 1-2. Predictions of weak link and strong link thermal tolerance responses to increasing and decreasing environmental variation over an evolutionary time scale

CHAPTER 2: MICROHABITAT AND BODY SIZE EFFECTS ON HEAT TOLERANCE

Previously peer reviewed and published journal article (Baudier *et al.* 2015).

Introduction

Predicting how animal populations and species respond to changing ambient temperatures is potentially complicated by the array of thermal microhabitats used by animals at any given site. Examples of microhabitat-driven thermal clines include those from canopy to forest floor (Hood & Tschinkel 1990; Weiser *et al.* 2010; Kaspari *et al.* 2015), patches of shade vs. sun (Huey *et al.* 1989; Meisel 2004; McGlynn *et al.* 2010), and the effect of soil thermal buffering relative to surface conditions (Zimmerman *et al.* 1994; Bulova 2002; Kumar & O'Donnell 2009). Current climate models rely largely on temperature data from weather stations placed 1–2 m above ground in the open (Hijmans *et al.* 2005; Strauss, Formayer & Schmid 2013). Standard weather data collection is inadequate for assessing the diversity of thermal environments animals experience within sites (Porter *et al.* 1973; Kaspari 1993; Meisel 2004; McGlynn *et al.* 2010; Andrew *et al.* 2013). Microhabitat-specific thermal tolerance data may be critical for understanding how terrestrial animal populations are adapted to and limited by temperature, and how they will respond to directional change (Huey *et al.* 2012). I suggest that thermally distinct microhabitats can select for different thermal physiologies among sympatric species within a taxon. My goal was to test whether species that use thermally distinct microhabitats differ in their upper thermal tolerance limits. As small-bodied, ecologically dominant ectotherms, ants serve as useful models for understanding responses to temperature variation and climate change (Harkness & Wehner 1977; Hölldobler & Wilson 1990; Andrew *et al.* 2013). Here, I develop and test the hypothesis that soil microhabitat use can predict species differences in the upper limits of thermal tolerance (maximum critical temperature, henceforth CT_{max}). I took advantage of the diversity of microhabitat use among Neotropical army ant species (Dorylinae) (Rettenmeyer 1963) to test whether microhabitat use predicts species differences in CT_{max}. Sympatric army ant species (co-

occurring at the same geographic site) range from largely above-ground activity to below-ground activity (Rettenmeyer 1963). An organism's thermal tolerance is the range of temperatures over which it maintains the capacity to function (Huey & Stevenson 1979; Angilletta, Niewiarowski & Navas 2002). Species and individuals vary in their thermal sensitivity, or the relative breadth of this thermal tolerance (Sunday, Bates & Dulvy 2011; Kaspari *et al.* 2015). CT_{max} is the maximum temperature at which an organism is able to function, setting the upper bounds of animals' thermal tolerance (Huey & Stevenson 1979; Ribeiro, Camacho & Navas 2012). CT_{max} is often close to optimum performance temperatures, particularly among thermal specialists with narrow thermal tolerance ranges (Sunday, Bates & Dulvy 2011; Huey *et al.* 2012). In the absence of other physiological data, CT_{max} can serve as an informative criterion for predicting species' geographic ranges, and for predicting costs associated with local temperature increases that result from climate change (Sunday, Bates & Dulvy 2011; Huey *et al.* 2012; Kaspari *et al.* 2015). I used species of New World army ants (Dorylinae), a monophyletic lineage, as subjects (Brady 2003; Brady *et al.* 2014; Borowiec 2016). I used the well-supported molecular-based genus level phylogeny to account for potential species relatedness effects on thermal physiology (Felsenstein 1985; Brady 2003; Brady *et al.* 2014). Neotropical army ant species share a combination of social, physiological and ecological attributes that make them tractable models for addressing microclimatic questions. All Dorylinae are top predators that conduct colony activities (foraging, colony migrations, and temporary nest formation) in massive coordinated social groups (Wheeler 1921; Schneirla 1933; Schneirla, Brown & Brown 1954; Rettenmeyer 1963). Importantly, species of Dorylinae vary in degree of subterranean activity (Rettenmeyer 1963; Kumar & O'Donnell 2009), enabling me to assess how variation in surface vs. subterranean activity corresponded to thermal tolerance. The nine army ant species assayed in this study represent a range from among the most subterranean to the most surface-active doryline species. I expected above-ground species would more often encounter extreme high temperatures that are potentially limiting to worker performance or survival, while the thermal buffering effects of subterranean microhabitats

would ameliorate selection for high-temperature tolerance. Therefore, I predicted the most above-ground species would have the greatest heat tolerances, with lower heat tolerance in ants that spend more time underground.

All army ant species have size-polymorphic workers, with some species overlapping widely in body size distributions (Rettenmeyer 1963; O'Donnell *et al.* 2011). This enabled within- and between-species analyses of body size effects on thermal tolerance (Clemencet *et al.* 2010; Oberg, Toro & Pelini 2012; Ribeiro, Camacho & Navas 2012; Kaspari 2013). Within-species body size variation is related to thermal tolerance in many ant species (Clemencet *et al.* 2010; Ribeiro, Camacho & Navas 2012): upper thermal tolerance limits (CT_{max}) generally increases with body size in ants, but the relationship between body size and CT_{max} varies substantially among ant taxa (Oberg, Toro & Pelini 2012). I asked whether microhabitat use was a better predictor of CT_{max} than body size for army ant species that overlapped in worker size distributions.

Methods

Army ant subjects

I collected and assayed army ants during the rainy season (June–August) of 2013 and 2014 in and around Monteverde, Costa Rica (N 10°18', W 84°48'). I conducted maximum thermal tolerance (CT_{max}) assays using 972 army ant workers collected from 47 colonies of nine species in three genera (Table 2-1). All species were previously described with the exception of one which keys to *Labidus praedator* (Smith) but is morphologically distinct in having a shining cuticle. These ants are strongly differentiated genetically from *L. praedator* and are broadly sympatric with *L. praedator* (Barth, Moritz & Kraus 2015). I treated them as a separate species, *Labidus* JTL001 (J T. Longino, pers. comm. 2014). Subjects were collected at elevations from 1283 m above sea level (m asl) to 1711 m asl in montane forests. At each army ant sample site, I recorded latitude,

longitude, and elevation using hand-held global positioning system (GPS) units. The GPS unit elevations were averaged with those generated from a digital elevation model (http://www gpsvisualizer com/elevation) to reduce error from either method. I located surface raids by walking cleared trails in search of ant columns (O'Donnell *et al.* 2007; Kumar & O'Donnell 2009; O'Donnell *et al.* 2011). When a column was encountered, I followed it in the opposite direction of portered food to reach the raid front. Whenever possible, I aspirated workers from raid fronts or raiding columns of ants near the front; some workers were also collected near bivouacs. Underground-raiding army ants were sampled via standard sunken traps baited with tuna oil (O'Donnell & Kumar 2006; O'Donnell *et al.* 2007; Kumar & O'Donnell 2009). A bulb planter was used to excavate a cylindrical hole 10 cm in depth and 10 cm diameter. Tuna oil was applied to the bare soil on the bottom of the hole so as to allow underground foragers to enter via tunnels from any side, including below. The hole was then covered with a 200-mL plastic cup and a plastic plate to create a darkened environment and to provide some protection from surface-foraging species. At each site, 5–10 baits spaced five meters apart were placed in a transect alongside permanent trails, 1–5m from the trail. Baits were checked every two to four hours until army ants were observed raiding in the trap pits.

Temperature measurements

I sampled soil temperatures at surface and 10 cm below the surface (the depth at which the ants were baited and collected). Observations of subterranean foraging depths for most army ant species are anecdotal and vary from several meters deep to beneath constructed soil tubes on the surface (Rettenmeyer 1963; Perfecto 1992). Foraging depths near the surface likely experience the most extreme fluctuations in diel temperature and humidity (Parton & Logan 1981). One to three pairs of thermal data loggers (ibutton; Maxim Integrated™, San Jose, CA, USA) were deployed along each bait transect at soil surface and 10 cm below the surface. Loggers recorded temperature every five minutes preceding, during and 3 days after n = 8 subsurface raid

collections (three raids in 2013, five raids in 2014). I used temperatures collected every five minutes across 24 h of each day within each site to calculate daily mean, maximum, minimum and range in temperature.

Surface raid temperatures at above-ground raids were collected using hand-held infrared thermometers in 2014 (BAFX Products, Milwaukee, WI, USA). All surface-accessible parts of 40 raids of five species were measured every 25 cm along the length of columns and across the width of raid fronts. All ibuttons were calibrated to within 0.5 °C (the manufacturer-reported instrument error). Ibutton accuracy was confirmed with a thermocouple, and also by water bath (0°C & 42°C as measured by a certified mercury glass thermometer). Infrared thermometer readings of soil temperatures were within 0.5 °C of temperatures recorded by ibuttons under field conditions.

Body size categories

I divided workers from each species into categories based on visually apparent body size and caste differences. In the case of *Eciton burchellii parvispinum* (Forel), the categories were as follows: soldiers, porters (sub-soldiers), large workers (media) and small workers (minima) (Franks 1989). For *Labidus spininodis* (Emery), *Neivamyrmex macrodentatus* (Menozzi) and *Neivamyrmex pilosus* (Smith), workers were placed into two categories: large and small. I divided all other species: *Eciton mexicanum* (Roger), *Labidus coecus* (Latreille), *L. praedator*, *L. JTL001*, and *Neivamyrmex sumichrasti* (Norton), into small, medium and large worker categories. After CT_{max} assays were complete, workers were stored together in 95% ethanol for later morphological measurements; workers from each size category per species were pooled. I photographed worker head capsules within each size category lying flat along the frontal plane using a digital camera (magnifications from 109 to 609, depending on head size) to generate 1200 x 900 pixel images. I then used IMAGE J software (version 1 45s; http://imagej nih gov/ij/) to measure head width at antennal insertions. I used the mean head capsule width of workers from each size category as a

standard index of body size (Burton & Franks 1985; Weiser & Kaspari 2006). Within each species, I conducted either a Student's t-test (species with two size categories) or an ANOVA with post hoc Tukey test (species with three or more size categories), using linear models in R, to confirm that the visually discerned body size categories differed significantly in head width. I used mean head capsule width for each worker category as the predictor variable in the analysis of CT_{max} differences among size categories.

Thermal tolerance assays

I used standard dynamic methods to measure each ant's critical thermal maximum (CT_{max}) (Lutterschmidt & Hutchison 1997; Diamond *et al.* 2012; Oberg, Toro & Pelini 2012). All assays were performed in a laboratory with ambient temperature similar to surrounding forest (in-laboratory temperatures recorded with ibuttons over two days: mean 21.05±2.50 °C SD, maximum 26 °C, minimum 17.5 °C). Time elapsed from collection to assay was recorded for each colony. Assays were conducted within 2 h of subjects being exposed to laboratory conditions, a standard practice that minimizes physiological acclimation to laboratory conditions (Oberg, Toro & Pelini 2012; Ribeiro, Camacho & Navas 2012), and within 5 h of subject collection from each raid site (mean 1.756 ± 0.878 h SD), to reduce effects of transit time. While in transit from the field, I supplied ants with water in excess (moistened paper towel) to prevent desiccation prior to performing the assays. Subject ants were individually placed into 1.5-mL plastic Eppendorf tubes stoppered with a cotton ball and then secured with a cap to prevent ants from seeking thermal refuge during the assay (Oberg, Toro & Pelini 2012). Approximately 20 vials with ants from each colony were then inserted simultaneously into a digitally controlled aluminum heat block which had been preheated to 30 °C. One ant from each caste in every colony was held in a similar vial adjacent to the heat block at laboratory ambient temperature as a control. I increased heat block temperature 1°C every 10 min, checking for movement at the end of each 10-min interval. The highest temperature at which an individual ant displayed movement

in response to light tapping was considered its CT_{max}. Immobility is a standard conservative measure of CT_{max} for ants (Lutterschmidt & Hutchison 1997; Meisel 2004; Oberg, Toro & Pelini 2012), and enabled better comparison across species than other measures.

Statistical analyses

Unless otherwise stated, all analyses were performed in R version 2 15 2 (R Development Core Team 2011)

Predictor variables of CT_{max}: I defined three categories of microhabitat use: 'above-ground' for species that bivouac and raid primarily above ground, 'below-ground' for those that typically raid and bivouac below ground and 'intermediate' for those that bivouac below ground and raid either partly or entirely above ground. However, the behaviors and activity budgets of some species are relatively poorly known, making precise categorization of microhabitat use difficult. As a proxy for microhabitat use, I developed a continuously varying morphological index of relative degree of above-ground activity for each species, the relative eye size index (RESI) (Figure 2-1):

RESI = external eye facet length/head width at antennal insertions

Eye size is an ecologically relevant morphological character that is predictive of environmental light exposure among many New World ant species (Weiser & Kaspari 2006). The army ant eye consists of a single highly reduced facet (omatidium), thought to mostly function to sense light levels (Rettenmeyer 1963). Increased ability to sense light may be more adaptive for surface-foraging army ants, which actively avoid forest clearings and sun flecks (Levings 1983; Meisel 2006). To control for variation in relative eye size among worker size-castes, I calculated the mean RESI value from the five smallest ants I collected in each species. Vestigial eye spots with no external lens were given an eye length of 0. RESI corresponded closely to the

microhabitat categories: more above-ground species generally had higher RESI values, with the most below-ground species having a RESI value of 0 (Figure 2-1). To check the validity of RESI as an indicator of habitat use, I ran all analyses (except the phylogenetic correction analysis) using habitat-use category in place of RESI and obtained similar results in all cases; I do not present the results of these analyses.

Testing predictors of CT_{max}: I used multifactor, mixed-model analyses of variance to identify significant predictors of CT_{max} (Quinn & Keough 2002). The full linear mixed-effects model (LMER in R) included the potentially confounding covariates: t-elapsed (time elapsed from collecting to assay) and elevation (metres above sea level) as fixed factors. RESI (microhabitat use index) and head width were also included as fixed factors. Colony was treated as a random nested variable within species. Effects of species and colony on head width distribution were included as random predictors of CT_{max}. I used restricted maximum likelihood and maximum likelihood estimations (via the ANOVA function) to identify and eliminate non-significant random and fixed variables, respectively. Sampling year effects on CT_{max} were tested by comparing the previously reduced model with and without year as a fixed variable for the subset of species sampled in both years. If a potentially confounding covariate had a significant effect, I included it in the reduced model; significance tests for the effects of predictor variables of interest account for these covariates.

Phylogenetic correction: The use of RESI as a continuously varying proxy for microhabitat allowed me to perform phylogenetic corrections. I used COMPARE software version 4 6b (Martins 2004) to analyze CT_{max} relationships with microhabitat with phylogenetic generalized least squares (PGLS) regressions based on Felsenstein's independent contrasts approach (Felsenstein 1985; Martins & Hansen 1997). COMPARE software version 4 6b was used (Martins 2004). I based the PGLS regression on the genus-level phylogeny of Dorylinae (Brady 2003; Brady *et al.* 2014) (Supplementary Figure 2-1). All branch lengths were set to one except branch lengths within species-level polytomies, which were set to a small value (0.001) as

recommended by Martins (Martins & Hansen 1997), this step was necessary because PGLS analysis requires a fully resolved phylogeny.

Temperature data: I compared daily maximum, minimum and temperature range differences between soil surface and 10-cm below ground using a linear mixed-effects model with transect as a random variable and above- vs. below-ground as a fixed-factor predictor variable

Results

Validating the methods

Head width differed significantly among my body size categories (ANOVA, $P < 0.0001$, $F_{24, 982} = 200.36$; this analysis included CT_{max} subjects and control ants). All size categories within each species differed significantly in head width (ANOVA + post hoc Tukey or Student's t-tests; all pairwise comparisons $P < 0.05$). However, there was considerable overlap in head size between species. 220 of 235 control ants (94%) survived in the laboratory throughout the duration of the assays, indicating holding ants in the assay tubes at ambient temperatures caused little mortality during the time required to assess CT_{max}.

Environmental temperatures

Surface and 10-cm subsoil probes differed significantly in all air temperature measures: average daily temperature (v2 = 224.7, df = 1, P =< 0.001), maximum daily temperature (v2 = 13.7, df = 1, P < 0.001), minimum daily temperature (v2 = 78.6, df = 1, P < 0.001) and daily temperature range (v2 = 56.1, d f. = 1, P < 0.001) (Table 2-2, Figure 2-2). Among all transects and days, air temperatures ranged at soil surface from 16 to 23 °C, with a range of 17.5–20 °C at depths of 10 cm. High temperatures recorded at 40 above-ground raids with IR thermometers were 36.1 °C for *N. sumichrasti*, 30 °C for *E. burchellii parvispinum* , 28 .8 for *L. praedator*, 20.1 °C for *L.*

JTL001 and 18.3 for *E. mexicanum* (Supplementary Table S2-1, Supporting Information). The most extreme surface temperature of 36.1 °C, measured at an *N. sumichrasti* raid, was greater than CT_{max} values measured for 11.83% of that species and exceeded CT_{max} for some individual workers in other species as well (Figure 2-3; Table 2-1; Supplementary Table S2-1)

Predictors of CT_{max}

CT_{max} values did not differ significantly between sample years among the four species sampled in both years (v2 = 3.544, df = 1, P = 0.060). Time elapsed between collection and assay was a significant predictor of CT_{max} (v2 = 6.12, df= 1, P = 0.013), so time to assay was corrected for in all statistical models. Both microhabitat use (as indexed by RESI) and body size were significant positive predictors of CT_{max} (Figures 2-5 and 2-6). CT_{max} was positively associated with colony differences in head width (v2 = 141.6, df= 3, P < 0.001) and species differences in head width (v2 = 24.5, df = 3, P < 0.001). Elevation was not a significant predictor of CT_{max} (v2 = 3.1, df = 1, P = 0.080). The interaction between microhabitat and head width was a significant predictor of CT_{max} (v2 = 4.047, df = 1, P = 0.044), indicating that smaller subterranean ants were most sensitive to high temperatures (Figure 2-5).

Phylogenetic correction

CT_{max} was positively associated with degree of aboveground activity after phylogenetic correction. RESI covaried positively with species mean CT_{max} (raw data R = 0.89, N = 9, P = 0.001; PGLS regression: R = 0.88, N = 8, P = 0.004; Figure 2-4).

Discussion

Microhabitat use and thermal tolerance

Species-typical microhabitat use (above- vs. below-ground activity) was highly significantly associated with species differences in heat tolerance (Figures 2-5 and 2-6). In general, CT_{max} was higher in the most above-ground species (*E. burchellii parvispinum*), lower in the most below-ground species (*L. coecus* and *N. macrodentatus*) and intermediate in the other sampled species which are active both underground and on the surface (Rettenmeyer 1963)(Table 2-1). This suggests greater heat tolerance is more adaptive for above-ground species due to higher maximum temperatures experienced in the surface environment.

Thermal tolerance generally increases with body size in ants (Cerdá & Retana 2000; Clemencet *et al.* 2010; Ribeiro, Camacho & Navas 2012). I showed that small workers were less heat tolerant in highly polymorphic army ants (Figure 2-5). Several physiological factors related to body size may affect size-caste differences in CT_{max}. Variation in heat-shock protein synthesis affected CT_{max} differences between highly thermophilic *Cataglyphis* species (Gehring & Wehner 1995). Differences in cuticular thickness may also be relevant, as reduced cuticle thickness is known to cause increased thermal conductance among insects (Galushko *et al.* 2005). The interplay between body size (surface area to volume ratio) and cuticular lipid content affects thermal tolerance in ants adapted to desiccation-prone microhabitats (Hood & Tschinkel 1990). CT_{max} increased with species mean body size in several ant subfamilies (Clemencet *et al.* 2010; Oberg, Toro & Pelini 2012; Ribeiro, Camacho & Navas 2012). My data suggest small army ant species were less able to survive extreme high temperatures than larger species. However, there was considerable overlap in body size categories between some army ant species I sampled, enabling comparisons of workers with similar body sizes across species. The effects of body size on maximum thermal tolerance were more extreme in more below-ground species. For example, small workers of the most subterranean species (*L. coecus* and *N. macrodentatus*) were less

thermally tolerant than expected from their body size relative to the other army ants I studied (Figure 2-5). This difference in the relationship between body size and CT_{max} among species indicates that high thermal tolerance in small workers is more adaptive in surface-dwelling species. Small ant workers reach equilibrium with ambient temperatures faster than larger nest mates; coupled with greater thermal sensitivity (lower CT_{max}), this suggests brief exposure to high temperatures is especially costly to small workers (Kaspari *et al.* 2015).

Environmental temperatures

I predicted that even a shallow soil depth would buffer thermal variation relative to the ground surface and that this selective pressure has resulted in higher CT_{max} among more epigaeic species. I found that 10 cm of soil caused significant thermal buffering at baits where below-ground raiding species were collected (Table 2-2, Figure 2-2). Although anecdotal reports suggest subterranean army ants such as *L. coecus* can forage much deeper (Longino pers. comm. 2014), even at this modest depth, workers experienced a distinct thermal environment that was moderated in temperature. In my ibutton sampling, below-ground daily average temperatures were slightly warmer than surface temperatures; however, maximum surface temperatures were significantly higher than those recorded underground, suggesting that occasional spikes in ambient temperatures aboveground may be a selective force on army ant CT_{max}. There was high temporal and spatial variability in surface temperatures experienced by above-ground and intermediate army ant species (those with medium to high RESI values). Maximum ground-surface temperatures recorded at a high-elevation raid of *N. sumichrasti* exceeded CT_{max} values for many small intermediate habitat-use army ants and some small workers of above-ground foraging species and exceeded CT_{max} of most individuals in below-ground species (Figure 2-3; Table 2-1). Although below-ground species are less likely to interact with these extreme temperatures, future increased incidence and duration of high surface temperatures are potentially limiting to species with intermediate and surface habitat usage patterns. It is important to note that

the criterion I used for exceeding CT_{max} (total immobility) is a conservative measure (Lutterschmidt & Hutchison 1997). Worker physiological impairment and colony fitness costs therefore may occur at temperatures below a species' CT_{max} as reported by this study. Deviations from optimum temperatures for tropical species with narrow thermal tolerances can impose high fitness costs even without causing observable mortality (Kingsolver, Diamond & Buckley 2013).

Ecological implications

I demonstrated that temperature differences caused by soil microclimates can select for species diversity in heat tolerance among montane Dorylinae. Population responses to directional climate change include shifts in latitudinal and elevational range (Colwell *et al.* 2008; Deutsch *et al.* 2008; Longino & Colwell 2011). However, patterns of range shifts can differ among species (Deutsch *et al.* 2008; Corlett 2011). Some of these species differences may be due to species-typical microhabitat use. I expect that future exploration of how populations differ across elevational gradients will show higher CT_{max} in lowland Dorylinae. This work is relevant to predicting potential for upward elevational shifts of army ant populations. I predict sympatric army ants (and other ectotherms) that occupy thermally distinct microclimates will experience different pressures as climate warms. Although climate change models predict less extreme temperature changes at low latitudes, the relatively narrow thermal tolerances among tropical species could exacerbate climate change impacts in the tropics (Deutsch *et al.* 2008; Sunday, Bates & Dulvy 2011; Chown 2012; Huey *et al.* 2012). I demonstrated that even at the highest elevations reported for surface-raiding army ants (over 1700 m a.s.l.), intermediate surface-raiding ants can encounter temperatures that challenge the thermal physiologies of their most sensitive castes. This indicates that a small directional change in climate may have a large impact on these species that already operate near the maximum of their thermal tolerance ranges. Army ants are abundant top predators that can strongly impact density, diversity and patchiness of their prey animals (Kaspari & O'Donnell 2003; Kaspari *et al.* 2011), and army ant colonies host

diverse symbionts (Rettenmeyer 1962; Rettenmeyer *et al.* 2011). My data suggest the effects of directional thermal change will vary among army ant species, potentially causing complicated alterations of forest community structure via effects on their prey and on their symbionts. CT_{max} is a commonly used measure of thermal sensitivity (Rezende, Tejedo & Santos 2011; Ribeiro, Camacho & Navas 2012). However, CT_{max} varied little along elevational and latitudinal thermal gradients relative to variation in minimum critical temperatures (CT_{min}) in diverse terrestrial ectotherms (Gaston & Chown 1999; Sunday, Bates & Dulvy 2011; Hoffmann, Chown & Clusella-Trullas 2013; Rezende, Casta~neda & Santos 2014). My findings show that CT_{max} can vary significantly on much smaller spatial scales, even among closely related taxa. This suggests that stability in thermally buffered soil temperatures vs. the heterogeneity and variability of surface temperature may play a key role in the evolution of heat tolerance in ectotherms that occupy these distinct microclimates.

Data accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/ 10.5061/dryad.7q3j8 (Baudier *et al.* 2015)

CHAPTER 2: TABLES

Table 2-1. Individual and colony sample sizes for CT_{max} assays for nine species in the genera *Eciton*, *Labidus* and *Neivamyrmex*; average and standard deviation of CT_{max} for each species; percentage of ants in each species found to be below the maximum raid temperature measured; ratio of eye to head width at antennal insertion (RESI) for smallest caste, a continuous variable used as proxy for soil microhabitat use; and species microhabitat use based on bivouacking and raiding above vs. below ground.

Species	**Worker N**	**Colony N**	**Mean CT_{max}**	**Median CT_{max}**	**CT_{max} SD**	**% CT_{max} < 36°C**	**RESI**	**microhabitat use**
E. b. parvispinum	359	17	40.20	40	1.528	1.11%	0.121	Above[a,b,c]
E. mexicanum	60	3	38.15	38	1.300	1.67%	0.139	Intermediate[b]
L. coecus	135	7	35.59	35	2.490	52.59%	0.052	Below[b,d,e]
L. praedator	59	3	36.39	37	2.385	32.20%	0.076	Intermediate[b,f]
L. JTL001	77	3	37.51	38	1.501	6.49%	0.072	Intermediate[g,h]
L. spininodis	19	1	35.37	35	1.535	57.90%	0.053	Intermediate[h]
N. macrodentatus	19	1	32.37	32	2.948	78.95%	0	Below[h,I,j]
N. pilosus	24	1	39.04	39	0.908	0%	0.103	Intermediate[b]
N. sumichrasti	220	11	38.24	38	1.449	6.36%	0.080	Intermediate[k]
Grand Total	**972**	**47**						

[a](Schneirla, Brown & Brown 1954), [b](Rettenmeyer 1963), [c](O'Donnell *et al.* 2011), [d](Kumar & O'Donnell 2009), [e](Perfecto 1992), [f](O'Donnell *et al.* 2007), [g](Pers. Comm, Longino 2014), [h](Pers. Obs.) [i](Menozzi 1931), [j](Watkins 1968), [k](Dunn 2003)

Table 2-2. Average daily maximum, minimum, mean and range in temperature (°Cc±SD) as recorded by ibutton thermal probes placed at soil surface and 10-cm subsurface across 8 bait transects sampled in 2013 and 2014

Soil depth	Max	Min	Mean	Range	N Days	N Points
0 cm	19.49±1.08	17.38±0.62	18.23±0.44	2.11±1.24	38	14216
10 cm	18.87±0.32	18.61±0.31	18.71±0.28	0.26±0.28	38	14217
Grand Total						**28433**

CHAPTER 2 FIGURES

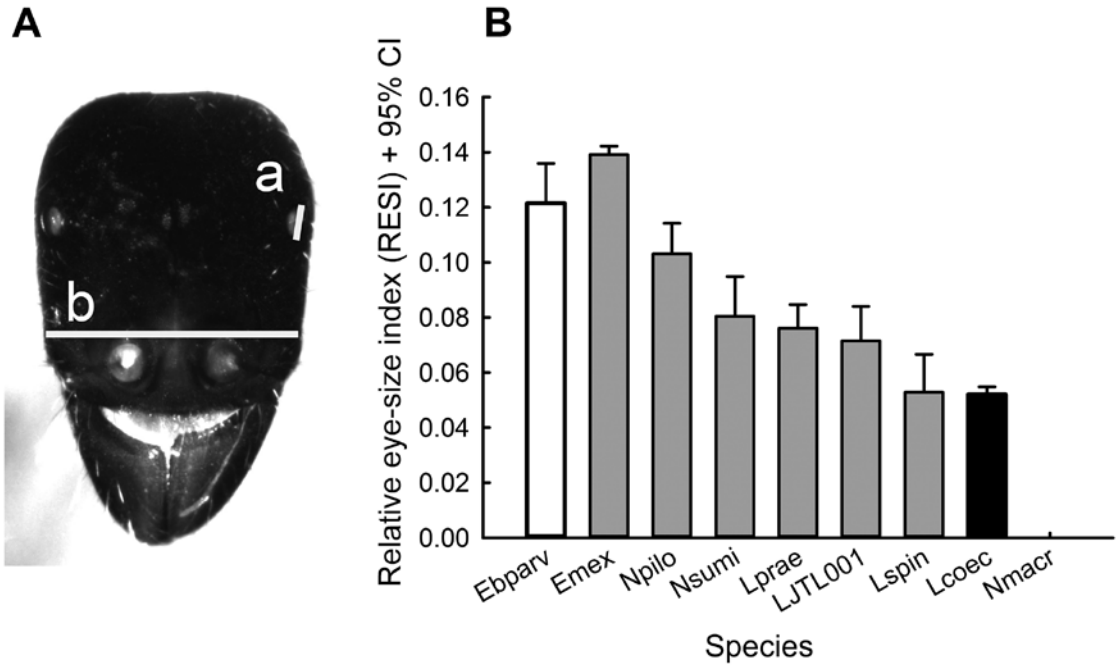

Figure 2-1. A. Frontal view of *Eciton burchellii* worker show characters used to estimated head with and relative eye-size index (RESI) = eye length (line a) / head width at antennal insertion (line b); **B.** RESI+95% confidence intervals in nine species of army ants with varying above and below-ground activity levels; white are species that typically bivouac and raid above ground, species shaded in black (*L. coecus* & *N. macrodentatus*) bivouac and raid below ground, and species in gray are intermediates

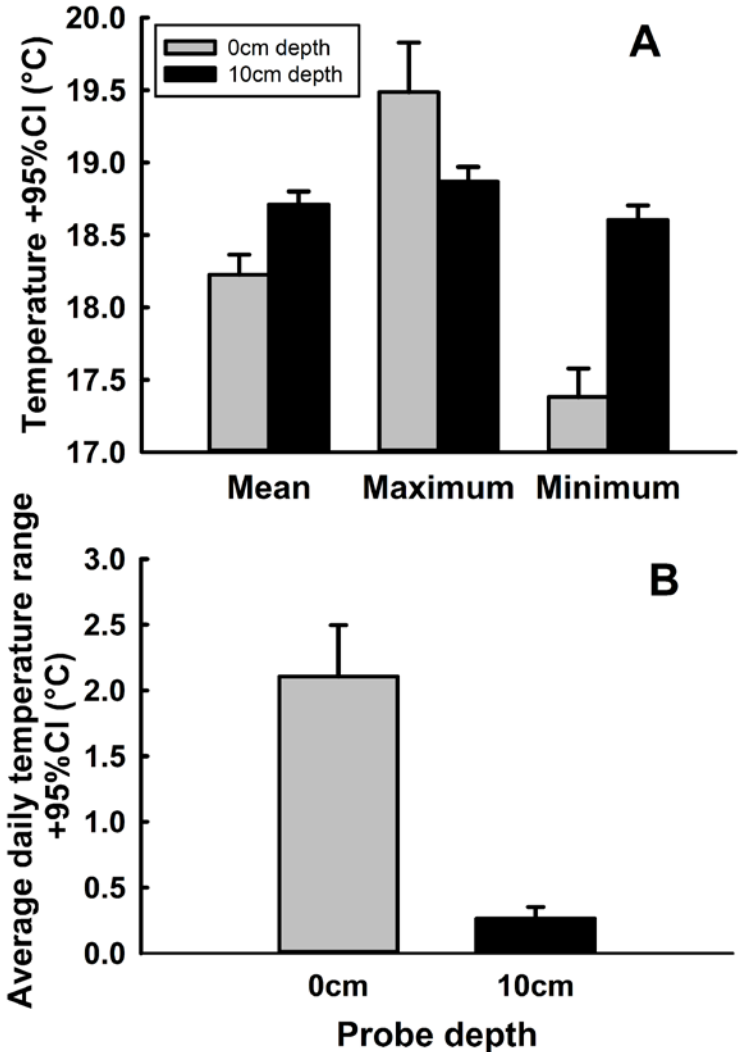

Figure 2-2. A. Average daily mean temperatures, daily maximum temperatures, and daily minimum temperatures with 95% confidence intervals, collected from temperature probes at two soil depths; **B.** Difference in average daily temperature range among two different soil depths

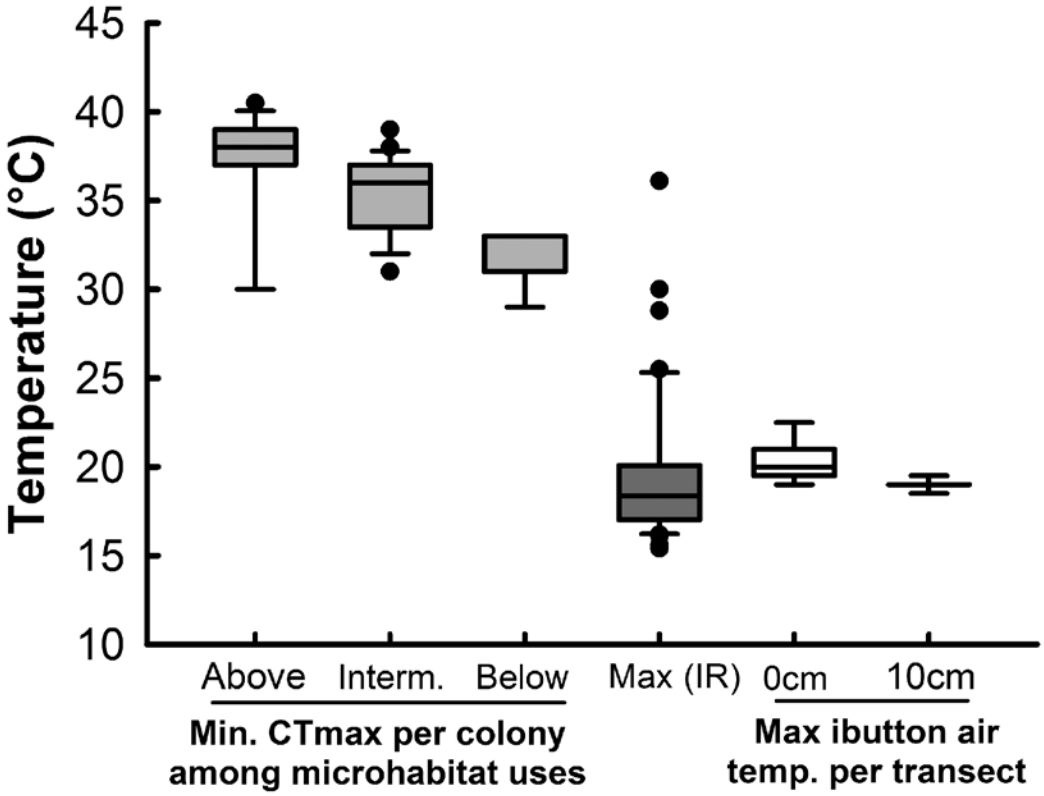

Figure 2-3. Proximity of CT_{max} values and two types of environmental temperature measurements: ibutton data-logger measurements and maximum infrared (IR) surface raid temperatures; light grey are minimum colony CT_{max} values for each habitat group, dark grey are maximum surface raid temperatures recorded, and white are ibutton bait trap temperatures recorded at soil surface and 10-cm depth.

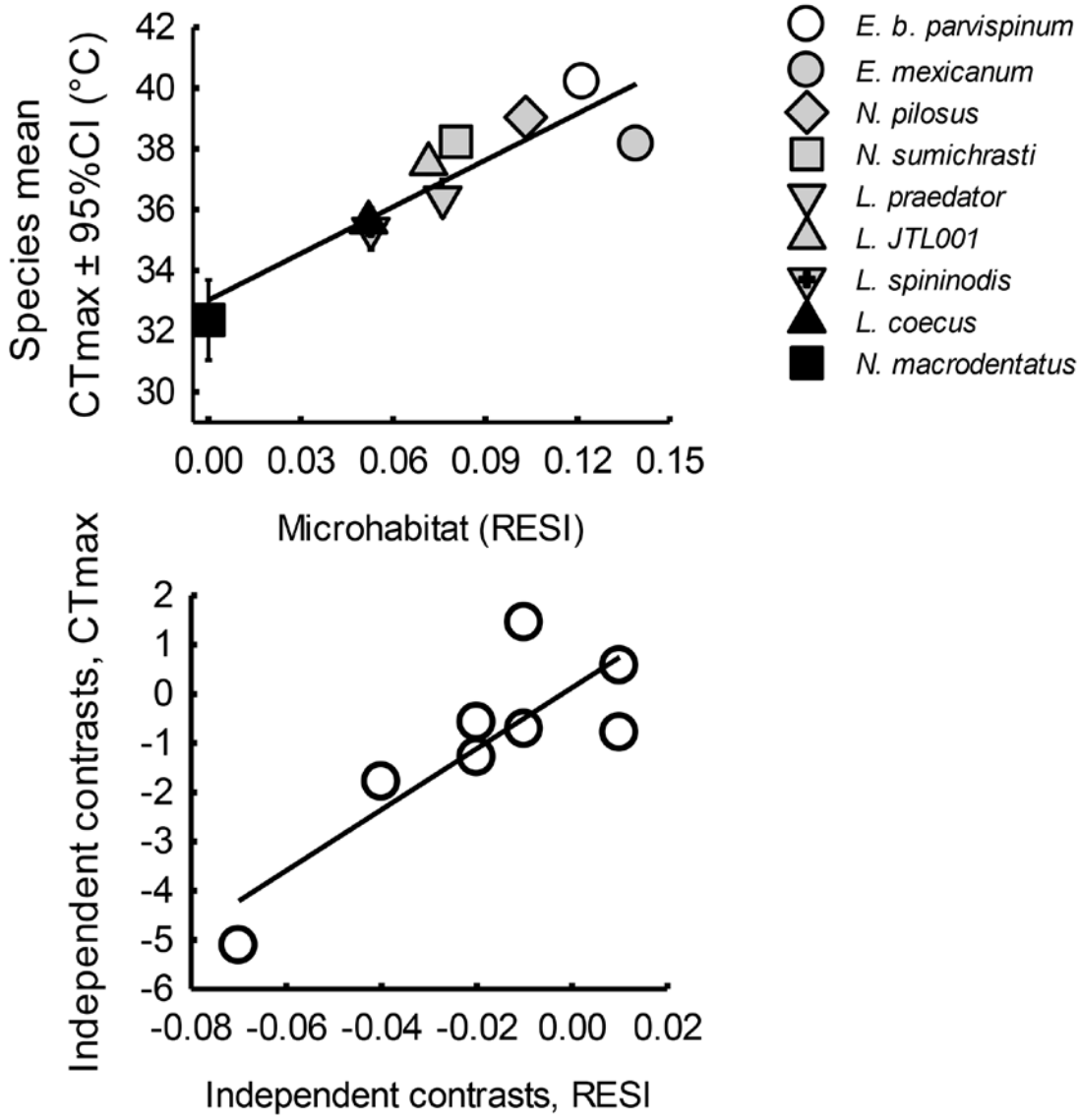

Figure 2- 4. A. Phylogenetically uncorrected correlation between average maximum critical temperature (CT_{max}) and relative eye size index (RESI) among nine species of army ants; white symbols indicate the species typically raid and bivouac above ground, black indicates raiding and bivouacking typically below ground, and grey indicates species that bivouac below ground but at least commonly raid above ground; circles are species of *Eciton*, triangles are species of *Labidus*, squares are species of *Neivamyrmex*; **B.** Independent contrasts showing significant (and similar) relationship between RESI and CT_{max} after accounting for relatedness

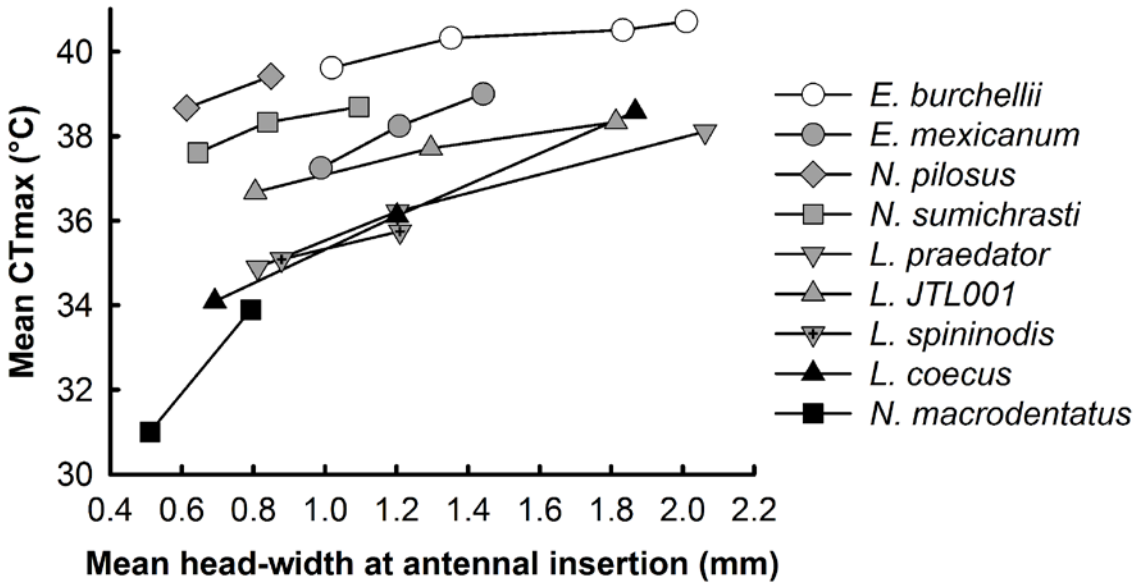

Figure 2-5. Relationship between CT_{max} and body size among nine species of army ants with three different uses of soil microhabitat; white symbols indicate the species typically raid and bivouac above ground, black indicates raiding and bivouacking typically below ground, and grey indicates species that bivouac below ground but at least commonly raid above ground; circles are species of *Eciton*, triangles are species of *Labidus*, squares are species of *Neivamyrmex*

CHAPTER 3: PHYSIOLOGICAL AND ECOLOGICAL EFFECTS ON THERMAL TOLERANCE ACROSS ELEVATIONS

Introduction:

The climatic variability hypothesis, or Janzen's rule, predicts thermal generalist species in environments with variable temperatures and thermal specialists in stable temperature environments (Janzen 1967; Addo-Bediako, Chown & Gaston 2000; Ghalambor *et al.* 2006; Gaston *et al.* 2009). Tests of this hypothesis have primarily focused on latitude, showing a general decrease in thermal tolerance breadth towards the equator (Deutsch *et al.* 2008; Sunday, Bates & Dulvy 2011). This pattern is caused by lowered minimum critical temperatures at high latitudes (CT_{min}) and relatively stable maximum critical temperatures (CT_{max}), a pattern known as Brett's rule or Rapoport's rule (Brett 1956; Stevens 1989; Gaston *et al.* 2009). However, due to the effects of topography, tropical sites at the same latitude can vary both in mean annual temperature and temperature seasonality (MacArthur 1972), providing ideal settings for independent tests of the underlying drivers of these patterns. Understanding the effects of local geographic variation on the evolution of thermal tolerance is important to predicting current distributions of populations and responses to climate change. I used elevationally wide-ranging army ants (subfamily Dorylinae) occupying a variety of microclimates to test for the effects of mean annual temperature, diel variation in temperature, and seasonality of temperature on thermal tolerances within a narrow range of tropical latitudes. I sampled thermal tolerance of army ant communities in forests that vary elevationally and in rainfall seasonality (from aseasonally wet with stable temperatures to seasonally dry with variable temperatures), while also taking into account species differences in soil microhabitat use and body size.

Soil microhabitat

New world army ants in the subfamily Dorylinae are tractable models for testing hypotheses relating to elevation and microhabitat as species differ in soil microhabitat use (surface vs. subterranean activity) spanning elevations from sea level to over 3000 meters above sea level (masl) (Pers. Com. John T Longino; (Kumar & O'Donnell 2009; O'Donnell *et al.* 2011). Temporary nests (bivouacs) of army ants in some species of *Eciton* are constructed entirely above ground (Schneirla, Brown & Brown 1954; Gotwald Jr 1995). Most other army ants bivouac underground (Rettenmeyer 1963), where they are shielded from diel extremes in temperature (Baudier & O'Donnell 2016). Army ant species also forage to different degrees above and below ground. Species such as *Eciton burchellii* (Westwood) and *Labidus praedator* (Smith) are generalist arthropod predators which raid across the surface of the forest floor in carpet swarms (Kaspari *et al.* 2011), while species such as *Labidus coecus* (Latreille) are subterranean predators of soil-dwelling arthropods including ground-nesting ants (Perfecto 1992). Soil buffering causes belowground foraging army ants to be shielded from extreme temperatures that surface-active species encounter, resulting in relatively high heat tolerance among aboveground army ant species (Baudier *et al.* 2015). However, the relationship between soil microhabitat use and either CT_{min} or thermal tolerance breadth is undescribed. I predict, consistent with the climatic variability hypothesis, that more subterranean species of army ants have narrower thermal tolerances and higher CT_{min} than surface-active species.

Elevation

The relationship between thermal tolerance and elevation is complex and has been most rigorously studied in non-tropical regions. Adiabatic cooling causes directional change in mean annual temperature —but not directional change in temperature variation— across elevation gradients (MacArthur 1972). Therefore, evolutionary responses to tropical elevational clines should involve adaptive changes in both thermal minima and maxima. Brett's rule suggests that

CT_{max} is less geographically variable than CT_{min} (Brett 1956; Gaston *et al.* 2009), but when applied to elevation this trend has mixed empirical support (Brattstrom 1968; Gaston & Chown 1999; Bishop *et al.* 2016) and contradiction (Karl, Janowitz & Fischer 2008; Oyen, Giri & Dillon 2016). This discrepancy represents a major source of uncertainty in the field of macrophysiology. Differences in diel thermal variation among spatial and temporal microclimates have been shown to be a strong selective force on CT_{max} as well as CT_{min} in the tropics (Baudier *et al.* 2015; Kaspari *et al.* 2015; Esch *et al.* 2017). I tested whether variation in species microhabitat use corresponds to different elevational patterns in thermal tolerance. I predicted that species with less exposure to diel temperature extremes, belowground army ants, adhere more closely to patterns predicted by adiabatic cooling (decrease in both CT_{max} and CT_{min} as elevation increases), as the buffered range of temperatures experienced underground are closer to mean ambient air temperature than above ground. Conversely, I predict less change in aboveground CT_{max} as surface-foraging army ants at high elevations are more likely to encounter rare but extreme high temperatures.

Thermal variation & rain shadows

Seasonal thermal variation is reduced in the tropics compared to temperate regions, but not all tropical regions are equally aseasonal. Seasonal changes in precipitation in tropical rain shadows can cause a spatial contrast in annual temperature variation across mountain ranges. Inhabitants of tropical seasonal dry forests experience changes in evaporative cooling, cloud cover, and often canopy cover at different times of the year, while rain forests on the prevailing side of mountain ranges do not experience these seasonal changes. In relatively aseasonal lowland Atlantic rain forests near La Selva Biological station, seasonal temperature change has been estimated to be less than 3°C (McDade 1994). In contrast, temperatures in the dry forests of Guanacaste (Pacific rain shadow) on average change 7°C at night and 12°C during the day across dry season versus wet season (Janzen 1986). Global Meta-analyses of squamate ectotherms have suggested that

both CT_{max} and optimum operative temperatures are more responsive to broad geographic patterns in thermal variation and precipitation than to mean ambient temperature (Clusella-Trullas, Blackburn & Chown 2011). Also, the climatic variability hypothesis predicts increased thermal generalism in more thermally variable environments. I therefore predicted that thermal tolerance breadth (primarily driven by changes in CT_{max}), would be greater in seasonal dry forests than in rain forests, independent of elevational effects.

Body size

Though thermal tolerance is often thought of as an individual physiological trait, the weak link hypothesis predicts individual thermal costs have colony-level effects in insect societies (Chapter 1; (Baudier & O'Donnell *In review*). Differences in metabolic rate, surface area to volume ratio, cuticular thickness, and thermal inertia are some explanations of the general trend for small-bodied castes within any given social insect colony to be more thermally sensitive to heat and cold than larger nestmates (Lighton & Bartholomew 1988; Clemencet *et al.* 2010; Ribeiro, Camacho & Navas 2012; Clarke, Thompson & Sinclair 2013; Wendt & Verble-Pearson 2016). However, depending on the thermal environment, these weak links may incur more colony-level costs than tolerant castes. In previous studies I have found that the effect of caste size on heat tolerance is reduced in more uniformly tolerant above-ground army ants (Baudier *et al.* 2015). I expected that selection acts against small sensitive castes of army ants more in thermally extreme aboveground habitats. I therefore predicted that small workers in above-ground species would be more thermally tolerant of extreme temperatures than the same size workers in below-ground species.

Acclimation

Improved thermal performance after a period of acclimation to new temperatures is a well documented adaptation to seasonal temperature variability (Jumbam *et al.* 2008; Modlmeier *et al.*

2012; Allen *et al.* 2016). Vernberg's rule suggests that acclimation is, for this reason, an adaptation primarily suited to species inhabiting high-latitude regions (Vernberg 1962; Gaston *et al.* 2009). Although many general principles of tropical physiological landscapes operate under this assumption (Ghalambor *et al.* 2006), whether and to what degree tropical species acclimate is less well known. Unlike most relatively immobile species of ants, army ant colonies are nomadic, relocating regularly across a landscape (Schneirla 1938; Rettenmeyer 1963; Gotwald Jr 1995; Soare *et al.* 2014). Though *Eciton burchellii parvispinum* (Forel) occurs as high as the continental divide in Costa Rica, it is also found in lowland seasonal dry forests near sea level, and northward as far as northern Mexico (Watkins 1976; Winston, Kronauer & Moreau 2017). I asked whether this thermally wide-ranging species showed signs of acclimation at either end of its elevational range in Costa Rica.

Methods

Field sites and sampling

Army ants were collected from six sites in northern Costa Rica. These sites varied in mean annual temperature due to adiabatic cooling across elevations, and in degree of climatic seasonality, from the relatively seasonal Pacific slope to the relatively aseasonal Atlantic slope of the continental divide due to effects of a rain shadow (Figure 1). Pacific slope sites consisted of lowland tropical dry forest and tropical moist forest in Santa Rosa National Park (N 10°53', W 85°46'), transitional premontane moist forest near Maritza Biological Station (N 10°58', W 85°30'), and premontane moist forest within San Luis Biological Reserve (N 10°15', W 84°50') (Janzen 1986; Nadkarni & Wheelwright 2000). Atlantic slope sites consisted of lowland tropical rain forest near La Selva Biological Station (N 10°25', W 84°01') and premontane rain forests near San Gerardo Biological Station (N 10°21', W 84°47') (McDade 1994; Nadkarni & Wheelwright 2000). Premontane and montane moist and wet forests were sampled in the area of Monteverde (N

10°18', W 84°49), which is predominantly on the Pacific side of the continental divide, but also includes sampled portions of the continental divide (Nadkarni & Wheelwright 2000). Santa Rosa was sampled from 3–31 May 2016; Maritza was sampled 16–18 May 2016; the San Luis Reserve was sampled 31 July 2014 and 3 August 2014; Monteverde was sampled 8 July–4 August 2014, and 6 April–1 May 2016; San Gerardo was sampled 22–24 March 2015 and 21–24 April 2016; and La Selva Biological station was sampled 17 June–5 July 2014. These collections include all 2014 samples from Monteverde which comprised half of the data set for Chapter 2 (Baudier *et al.* 2015).

Within each site standard daily trail walking was used to locate surface-foraging army ant species, while subterranean foraging species were baited using tuna oil in bottomless covered pitfall traps (Meisel 2006; O'Donnell *et al.* 2007; Kumar & O'Donnell 2009; O'Donnell *et al.* 2011; Baudier *et al.* 2015). Each tuna bait was10 cm deep. Transects consisted of 5 baits spaced 5 m away from each other and >3 m away from foot trails or roads. When possible, encountered army ant columns and raids were followed back to bivouacs, and bivouacs tracked over time in order to delineate colonies within each site. In cases of inaccessible bivouacs, encounters of conspecific raids greater than 300 m apart within 24 hours were assumed to be different colonies. Sampling for thermal tolerances consisted of N=3988 worker ants of N=128 colonies of N=15 army ant species (Table 1). Once collected, ants were provided a moistened paper towel to prevent desiccation and transported live back to the lab. Thermal tolerance assays began within 3 hours of collection. Coordinates and elevation at the site of each army ant colony's raid were recorded using hand-held GPS units (Garmin CPSMAP® 62S, Garmin International Inc., Olathe, KS, USA). Voucher specimens of each species were deposited into the entomology collection of the Academy of Natural Sciences of Drexel University.

Climate data

Three types of climatic data are compared to thermal tolerance: temperatures collected from continuous temperature loggers, temperatures gathered using infrared thermometers at sample sites, and extrapolated bioclimatic (bioclim) estimates from 1960–1990. At the site of each tuna trap two pairs of thermochron ibutton data loggers (Maxim Integrated™, San Jose, CA, USA) were deployed, recording temperature at the soil surface and 10 cm below the surface every five minutes for 3 consecutive days. These temperatures were used to compare diel variation in temperature above and below ground across sites, with the exception of the San Luis reserve, as overnight access was not possible in this sampling site. A subset of 10 cm depth ibutton data one hour before and after subterranean ants were observed at baits was used to estimate subterranean raid temperatures. Surface raid temperatures were recorded across elevations and sites using hand-held infrared thermometers (BAFX Products, Milwaukee, WI, USA). Consecutive infrared surface temperatures were taken 25 cm apart along the portion of the column and raid front which was exposed to the surface. Elevations used in this study were gathered by hand-held GPS units, while temperature annual range (maximum temperature of hottest month – minimum temperature of coldest month) data for 1960–1990 was extracted from bioclim using coordinates of army ant raids via the geographic coordinate system WGS1984 at a resolution of 1 km^2 (Hijmans *et al.* 2005; Hijmans *et al.* 2008).

Species soil microhabitat use

Army ant species used in this study were: *E. burchellii parvispinum, Eciton burchellii foreli* (Mayr), *Eciton hamatum* (Fabricius), *Eciton lucanoides* (Emery), *Eciton mexicanum* (Roger), *Eciton vagans* (Olivier), *Labidus coecus* (Latreille), *Labidus* JTL001 (Barth, Moritz & Kraus 2015), *L. praedator*, *Labidus spininodis* (Emery), *Neivamyrmex gibbatus* (Borgmeier) *Neivamyrmex macrodentatus* (Menozzi), *Neivamyrmex pilosus* (Smith), *Neivamyrmex sumichrasti* (Norton), and *N. esenbeckii*. An average index of surface activity (ISA) was calculated for each species by z-transforming and averaging the literature-based index of army

ant surface activity (IL) by Bulova *et al.* (2016) and the relative eye size index (RESI; small worker proportion of eye height to head width at antennal insertion) by Baudier *et al.* (2015) as follows where σ is the standard deviation across species:

$$ISA = \frac{\frac{IL - \overline{IL}}{\sigma_{IL}} + \frac{RESI - \overline{RESI}}{\sigma_{RESI}}}{2}$$

ISA is therefore a normalized index equally based on two independent measures of surface activity levels in army ants: prior description of behavior in well-studied taxa, and morphological adaptation to light exposure. Using this method better characterizes species that are less well-described behaviorally and reduces the effect of large eye facet bias among nocturnal species such as *N. gibbatus* and *E. mexicanum* (Moser *et al.* 2004). ISA is used as a proxy for species differences in soil microhabitat for all analyses.

Thermal tolerance assays

Thermal tolerance assays were conducted using the standard dynamic method of thermal ramping for both upper and lower critical limits (Lutterschmidt & Hutchison 1997; Oberg, Toro & Pelini 2012; Bishop *et al.* 2016). Ants were placed individually in 1.5 ml conical tubes stoppered with cotton to prevent access to thermal refuges at the top of the tubes. Depending on space in thermal blocks and number of ants collected, 14 to 20 ants from each colony were placed in either CT_{max} or CT_{min} assays. A dry heat block (Thermal-Lok 1 & Thermal-Lok 2, USA Scientific, Orlando, FL, USA) was used for CT_{max} assays and a Tropicooler™ (TropiCooler™ Benchtop Hot/Cold Block Incubator, Boekel Scientific, Feasterville, PA, USA) was used for CT_{min} assays. CT_{min} blocks were pre-cooled to 15°C, while CT_{max} blocks were pre-heated to 30°C at the start of each assay. Ants were exposed to incrementally more extreme temperatures at a rate of 1°C every 10 minutes. At the end of each 10-minute interval, subjects were checked for movement in response

to light tapping, as observed for 10 s after removal from blocks. CT_{max} was considered to be 1°C less than the temperature at which an individual lost mobility response in heat tolerance assays. CT_{min} was defined as 1°C greater than the temperature at which an individual lost mobility response in cooling assays. Colony thermal tolerance breadth was the mean CT_{max} – mean CT_{min} per colony sampled. Five ants per colony ranging in apparent size and cast were placed in Eppendorph tubes stoppered with cotton, but were kept on the side, to test for possible unexpected sources of colony mortality such as illness or inadvertent injury during transport. Any colony with more than 2/5 control mortality before the end of each thermal tolerance assay was not included in this study. This occurred only twice. Across all performed assays, 99% of control ants survived.

Body size measurements

Each subject ant was photographed with a dissecting-scope mounted digital camera (10 MP USB2.0 Microscope Digital Camera, Amscope, Irvine, CA, USA). Individual head width at antennal insertion was measured using ImageJ software (https://imagej.nih.gov/ij/) and was used as a proxy for body size in all analyses. Head width at eye height was not used due to the absence of eyes in some species of army ant. Relative eye height to head width at antennal insertion was also measured in this manner among the five smallest-caste workers within each species. This number was used to calculate ISA as mentioned previously.

Acclimation

We tested for acclimation capacity in the most elevationally wide-ranging aboveground species (*E. burchellii parvispinum*). Thermal tolerances of workers assessed within 2 hours following collection were compared to thermal tolerances of those subjected to 24 hours of acclimation at either 32°C or 8°C incubation temperatures. During periods of incubation ants were provided with water in excess (moistened paper towel), and relative humidity was kept between 80% and

95% to prevent desiccation. Both CT_{max} and CT_{min} were performed for non-acclimated, cold-acclimated and warm-acclimated groups. This was done both at the high-elevation range extent in Monteverde (~ 1600 masl) and at the low elevation range extent in Santa Rosa National Park (~ 300 masl). Two colonies of *E. burchellii parvispinum* were assayed at each site. N=480 worker ants in total comprised the subjects for this study.

Statistical analyses

All analyses were performed in R version 3.1.2 (R core team 2014).

To test for the effects of site and soil depth on ambient temperature, bait ibutton means and temperature ranges across all sampled days were set as response variables in two separate linear mixed model analyses. These mixed model analyses took into account transect and bait ID as random variables and site and soil depth (0 cm versus 10 cm) as fixed predictors. Predictor variables were eliminated until the minimum required model was reached, as recommended by Quinn & Keough (2002). Akaike information criteria (AIC) for each variable informed order of elimination comparisons. Infrared-recorded temperatures of surface raids were analyzed in a separate, similar mixed model analysis, which included site and species as fixed predictor variables and raid ID as a random variable.

New world army ants are monophyletic with a well-supported genus-level phylogeny (Brady 2003; Brady *et al.* 2014; Borowiec 2016) and recent further resolution within *Labidus* and *Eciton* (Barth, Moritz & Kraus 2015; Winston, Kronauer & Moreau 2017; Figure 2). Phylogenetically independent contrasts were calculated from army ant species mean values for ISA, CT_{max}, CT_{min}, and thermal tolerance breadth (Felsenstein 1985) using the ape package (Paradis, Claude & Strimmer 2004). Branch lengths were set to 1, with the exception of a Polytomy in the genus *Neivamyrmex*, for which branch lengths were set to 0.001, as recommended by Martins & Hansen (1997). ISA was regressed against each of these three

response variables. Significance and slopes were compared between corrected and non-corrected species level regressions.

To analyze effects of size and climate on thermal tolerance among army ant species, linear mixed models were fitted with CT_{max} as a response variable; fixed predictors were ISA (microhabitat use), head width (body size), elevation, annual range in temperature, ISA / head width interactions, ISA / elevation interactions, and ISA / annual temperature range interactions; and random variables were colony ID, site (Santa Rosa, San Gerardo, San Luis, Monteverde, Maritza, or La Selva), and taxon (species or subspecies as depicted in Figure 2). Predictor variables were eliminated until the minimum required model was reached. Akaike information criteria for each variable informed order of elimination comparisons. The equations of these reduced models were used to calculate all trends. An identical but separate analysis was conducted on CT_{min} data. The effects of these predictors on mean colony thermal tolerance breadth (mean CT_{max} – mean CT_{min}) were analyzed in a separate analysis that did not account for random effects of colony.

To test for acclimation effects in *E. burchellii parvispinum*, linear subset models were fitted with head width, treatment and colony ID as fixed predictor variables and either CT_{max} or CT_{min} as the response variable, eliminating predictor variables until the minimum required model was reached. Akaike information criteria for each variable informed order of elimination comparisons. This was done separately for high and low elevations. Post-hoc Tukey tests were used to compare high, low, and no acclimation treatment groups.

Results

Microclimate temperatures

Mean temperature differed significantly across sites as measured by ibuttons at baits (X^2=103.68, df=4, $p<0.001$) and IR temperatures at surface raids (X^2=128.89, df=5,8, $p<0.001$; Figure 1).

Surface raid temperatures did not differ across species (X^2=14.35, df=12,20, p=0.279). Mean continuous probe-recorded temperature was the same at soil surface and 10 cm below ground (X^2=0.016, df=1,8, p=0.900), but temperatures 10 cm below soil surface were less variable than surface temperatures, having on average 4.6°C smaller temperature range than above ground (X^2=25.485, df=1,8, p<0.001; Figure 1). Diel temperature range as measured by ibuttons also varied across sites (X^2=18.397, df=4,8, p=0.001) with Santa Rosa having an average temperature range of 7.3°C greater than La Selva (t=2.11, z= 3.44, p= 0.005) and 6.5°C greater than Monteverde (t=1.60, z=4.037, p < 0.001).

Phylogenetically independent contrasts

The effect of surface activity on species mean thermal tolerance breadth and CT_{max} was the same with and without correcting for relatedness (Figure 3). Thermal tolerance breadth increased with degree of above-ground activity using raw data ($F_{1,13}$=16.05, R^2=0.518, p=0.001; y=31.50+2.61x; slope SE=0.60) and independent contrasts ($F_{1,12}$=5.756, R^2=0.268, p=0.034; y=-0.46+2.46x; slope SE=1.02). CT_{max} also increased with ISA in both uncorrected data ($F_{1,13}$=18.968, R^2=0.562 p=0.001; y=37.84+1.74x; slope SE=0.40) and when accounting for relatedness ($F_{1,12}$=8.419, R^2=0.363, p=0.013; y=-0.34+1.72x; slope SE=0.59). The negative relationship between CT_{min} and ISA was less strongly significant using uncorrected data ($F_{1,13}$=6.05, R^2=0.265, p=0.029; y=6.29-0.83x), and this relationship became non-significant when accounting for relatedness ($F_{1,12}$=2.21, R^2=0.09, p=0.163).

Predictors of thermal tolerance

Fitted equations of thermal tolerance metrics and their bioclimatic and physiological predictors are as follows where E is elevation, W_H is individual ant head width, $\overline{W}_H$ is colony mean head width, T_{AR} is annual temperature range, and $\overline{Br}_{col}$ is colony mean thermal tolerance breadth:

$$CT_{max} = 31.20 - 3.46 \cdot 10^{-4}(E) + 1.46(W_H) - 1.69(ISA) + 0.44\,(T_{AR}) - 0.62(\mathrm{ISA} \cdot W_H) + 6.23 \cdot 10^{-4}(ISA \cdot E)$$

$$CT_{min} = 7.28 - 7.45 \cdot 10^{-4}(E) - 0.61(W_H) - 1.78(ISA) + 0.73(\mathrm{ISA} \cdot W_H)$$

$$\overline{Br}_{col} = 29.01 - 3.52 \cdot 10^{-4}(E) - 2.79(\overline{W}_H) - 0.89(ISA) + 1.31(\mathrm{ISA} \cdot E)$$

These equations were based on tests of significance and effect value estimates and were used to generate trends in all subsequent graphs that include thermal tolerance metrics as response variables.

Elevation & soil microhabitat

The effect of elevation on CT_{max} differed by soil microhabitat use: subterranean ants decreased in CT_{max} as elevation increased while more surface-active species remained relatively stable across elevations (X^2=4.86, df=1,10, p=0.027; Figure 4). Across all army ant species CT_{min} decreased as elevation increased (X^2=8.62, df=1,7, p=0.003; y=6.48-0.000745x; Figure 4). Surface-active species had lower CT_{min} than subterranean species within each elevation, but the elevational effect on CT_{min} did not significantly vary across microhabitats (X^2=2.39, df=1,9, p=0.122). Colony mean thermal tolerance breadth increased with index of surface activity, but the magnitude of this effect was greater at high elevations than in the lowlands; below-ground species decreased in thermal tolerance breadth across elevations while others did not (X^2=5.69, df=1,7, p=0.017; Figure 4).

Seasonality

CT_{max} was significantly higher in environments with higher variation in annual temperature (X^2=12.348, df=1,10, p<0.001; Figure 5). However, annual temperature range was not a significant predictor of CT_{min} (X^2=3.173, df=1,8, p=0.075) or colony mean thermal tolerance breadth (X^2=0.002 df=1,8, p=0.9629).

Soil Microhabitat & Body size

CT_{max} increased with body size, but subterranean species showed a more pronounced effect (X^2=57.522, df=1,10, p<0.001; Figure 6). CT_{min} decreased as body size increased, and this effect was more pronounced in belowground species (X^2=40.154, df=1,7, p<0.001). More surface-active species had larger thermal tolerance breadths, but this effect was more pronounced at high elevations than in the lowlands ($X^{2=}$5.694, df=1,7, p=0.017). The effect of microhabitat on CT_{max} was also greater at high elevations (X^2=4.864, df=1,10, p=0.027). Colony head width range was not a significant predictor of colony thermal tolerance breadth (X^2=0.213, df=1,11, p=0.645), but thermal tolerance breadth increased as mean colony head width increased (X^2=5.850, df=1,7, p=0.01558).

Acclimation

In Monteverde *E. burchellii parvispinum* incubated at either 32°C or 8°C for 24 hours performed the same as non-acclimated nestmates in both CT_{max} assays ($F_{2,116}$=0.32, p=0.730) and CT_{min} assays ($F_{2,93}$=0.82, p=0.442). In Santa Rosa acclimation treatments differed in CT_{max} ($F_{2,118}$=5.08, p=0.008) and CT_{min} ($F_{2,104}$=44.157, p<0.001), however non-acclimated ants performed better than acclimated ants. In Santa Rosa non-acclimated ants had lower CT_{min} than either heat-acclimated (t=9.32, p<0.001) or cold-acclimated nestmates (t=5.18, p<0.001), while non-acclimated ants had the same CT_{max} as cold-acclimated nestmates (t=0.64, p=0.801) and higher CT_{max} than heat-acclimated nestmates (t=3.03, p=0.008).

Discussion

Temperature data

In general, as expected higher elevations were cooler due to adiabatic cooling. However, bioclim data from 1960 to1990 were not in total agreement with ibutton or IR temperatures in Santa Rosa

and La Selva. This is likely partly due to differences in data collection methods, including that ibutton and IR temperatures reported in this study do not represent year-round sampling. Also, from late 2014 to early 2016 effects of an abnormally strong and long-lasting El Niño caused historically high rains in the Atlantic lowlands and historically dry and hot weather in Guanacaste. This too is likely part of the discrepancy.

Soil microclimate and thermal tolerance

Our findings on the microhabitat scale supported the climatic variability hypothesis, showing increased thermal tolerance breadth among surface-active species, which encounter more diel variation in temperature. Although Bret's rule suggests that CT_{min} is more geographically responsive than CT_{max} (Brett 1956; Gaston *et al.* 2009), I found the converse pattern on the scale of soil microclimate. Soil microhabitat use was a significant predictor of both CT_{max} and CT_{min}, but a stronger relationship was observed in CT_{max}.

Elevation

Although both subterranean and aboveground species similarly decreased in CT_{min} towards high elevations, CT_{max} response to elevation was dependent upon species microhabitat use. As predicted, subterranean species of army ant decreased in CT_{max} while surface-active species stayed relatively stable. The confounding variable of microhabitat, if not taken into account, would have masked elevational effects on CT_{max}, though not on CT_{min}. Microhabitat sampling bias is therefore a potential major source of error for studies that characterize large geographic patterns in CT_{max} because aboveground species fit the pattern of Brett's rule, while subterranean species fit the prediction of mean temperature-driven CT_{max} and CT_{min}.

Elevational effects in CT_{max} being more pronounced in subterranean species may be due to subterranean species experiencing temperatures which, even at their most extreme below ground, are always relatively near to mean annual temperature, while aboveground species may

be selected upon more heavily by rare but extreme temperatures. Meta analyses have previously suggested that CT_{max} varies globally with maximum temperature extents more than with mean temperature differences, as seems to be the case with CT_{min} (Clusella-Trullas, Blackburn & Chown 2011). My findings support this hypothesis over those that suggest general geographic uniformity in CT_{max} compared to CT_{min}, as CT_{max} varied more than CT_{min} with elevation for the most subterranean species in this study.

The magnitude of elevational change in CT_{min} and CT_{max} for subterranean species was only about one tenth that predicted by adiabatic cooling: 9.8°C over 1000 m asl (Lazaridis 2011). This is less than reported elevational change in CT_{min} in other taxa (Gaston & Chown 1999), though consistent with slopes of elevational change for CT_{min} in other studies using ants as models (Bishop *et al.* 2016).

Body size & Soil microclimate

The significance of the interplay between body size and soil microhabitat use is consistent with previous findings on a smaller subset of army ant species at high elevations (Chapter 2; Baudier *et al.* 2015). However, here for the first time I demonstrate these patterns in fine resolution. Thermal sensitivity among small workers differed more dramatically with soil microhabitat use. Highly surface-active species not only had wider thermal tolerance breadths, but also had less variation in thermal tolerance among ants of different sizes, due largely to broader small worker thermal tolerances. Furthermore, the thermal tolerance breadths of the most sensitive castes correspond closely to the ranges of temperatures recorded at raids in this study. These patterns support the weak link hypothesis for thermal tolerance in social insects, which predicts a disproportionate amount of selective pressure on these “weak link” (highly sensitive) individuals in environments with high thermal variation (Baudier & O’Donnell *In review*; Chapter 1).

Seasonality & thermal tolerance

CT_{max} increased with annual temperature range. These findings, like those for soil microhabitat, support the climatic variability hypothesis. Importantly, this also suggests that, among already thermally sensitive tropical species, those inhabiting lowland wet forests may be especially susceptible to directional change in temperature. This pattern is very similar to that of latitude: low variability habitats select for low-tolerance individuals (Sunday, Bates & Dulvy 2011), except that CT_{max} rather than CT_{min} is the major pattern-driver. This pattern of temperature variability and CT_{max} was predicted by global meta-analyses (Clusella-Trullas, Blackburn & Chown 2011), but this is the first test of this on the scale of tropical local rain shadows.

Acclimation

We observed no evidence of beneficial acclimation response. These findings did not support the hypothesis of acclimation as an adaptation to cross-elevational epigaic nomadism in *E. burchellii parvispinum.* This was, however, consistent with an observed lack of acclimation response in *L. praedator* (Baudier & O'Donnell 2016) and ultimately supports Vernberg's rule, which predicts lack of acclimation capacity among tropical species (Vernberg 1962; Gaston *et al.* 2009). These findings offer tentative support for the possibility that fixed genetic differences among high and low elevations may account for within-species differences in thermal tolerance across elevation ranges.

In conclusion

Thermal tolerance varied with adiabatic cooling, rain shadows, and local microclimates. Due to differences in environmental temperature variability, microclimate use modified selective pressure along tropical elevation gradients, and across body sizes of sympatric species. Although CT_{min} has been widely held as the more climate-responsive thermal tolerance limit, I demonstrated stronger patterns in CT_{max} than CT_{min} in response to soil microhabitat, body size,

and annual temperature range within the tropics. These findings establish new connections between macrophysiology and microclimate, with the goal of expanding understanding of temperature as an evolutionary selective force and improving predictions of climate change impacts on a global scale.

Table 3-1. Degree of aboveground activity of different army ant species; average index of surface activity (ISA) was calculated to be the z-transformed literature-based index (IL) used by Bulova *et al.* (2016) averaged with z-transformed (RESI) by Baudier *et al.* (2015); lower scores correspond to more subterranean species

Species	**N colonies**	**N ants**	**Raid**	**Bivouac**	**IL**	**RESI**	**ISA**
Neivamyrmex macrodentatus	1	33	1	1	2	0.00	-1.94
Labidus coecus	9	312	1	1	2	0.07	-1.10
Nomamyrmex esenbeckii	2	80	2	1	3	0.07	-0.88
Labidus spininodis	3	92	3	1	4	0.07	-0.67
Labidus JTL001	2	92	4	1	5	0.07	-0.45
Labidus praedator	10	279	4	1	5	0.08	-0.33
Neivamyrmex sumichrasti	11	301	4	1	5	0.08	-0.33
Neivamyrmex pilosus	8	283	4	1	5	0.10	-0.09
Eciton lucanoides	2	108	4	2	6	0.13	0.48
Neivamyrmex gibbatus	1	78	4	1	5	0.15	0.51
Eciton mexicanum	7	229	4	2	6	0.14	0.60
Eciton vagans	16	483	5	2	7	0.13	0.70
Eciton burchellii parvispinum	37	1167	5	3	8	0.12	0.79
Eciton hamatum	6	235	5	4	9	0.13	1.13
Eciton burchellii foreli	5	216	5	5	10	0.15	1.59

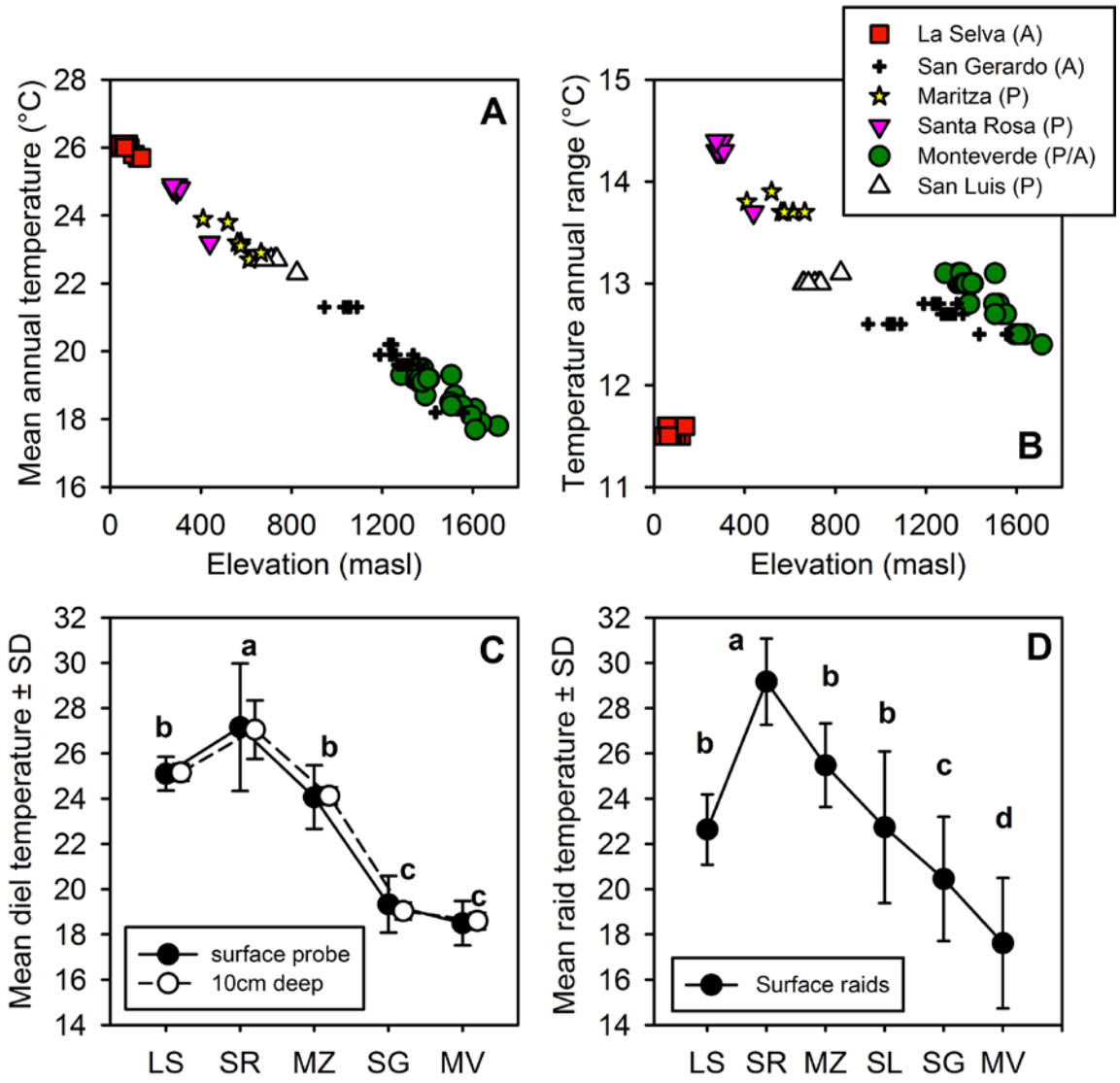

Figure 3-1. Overview of climate data for 6 Costa Rican sites used in the study (A) tight relationship between elevation and bioclim 1960-1990 estimates for mean annual temperature (B) disassociation between elevation and bioclim 1960-1990 estimates for temperature annual range, between sites on the Pacific slope of the continental divide (P) and the Atlantic slope of the continental divide (A); (C) mean temperatures and variance of temperatures as measured by ibuttons placed on the forest floor versus at tuna oil baits 10 cm underground, letters denote significant differences among site means; (D) mean and standard deviation of surface raid temperature at each site, letters denote significant differences among site means

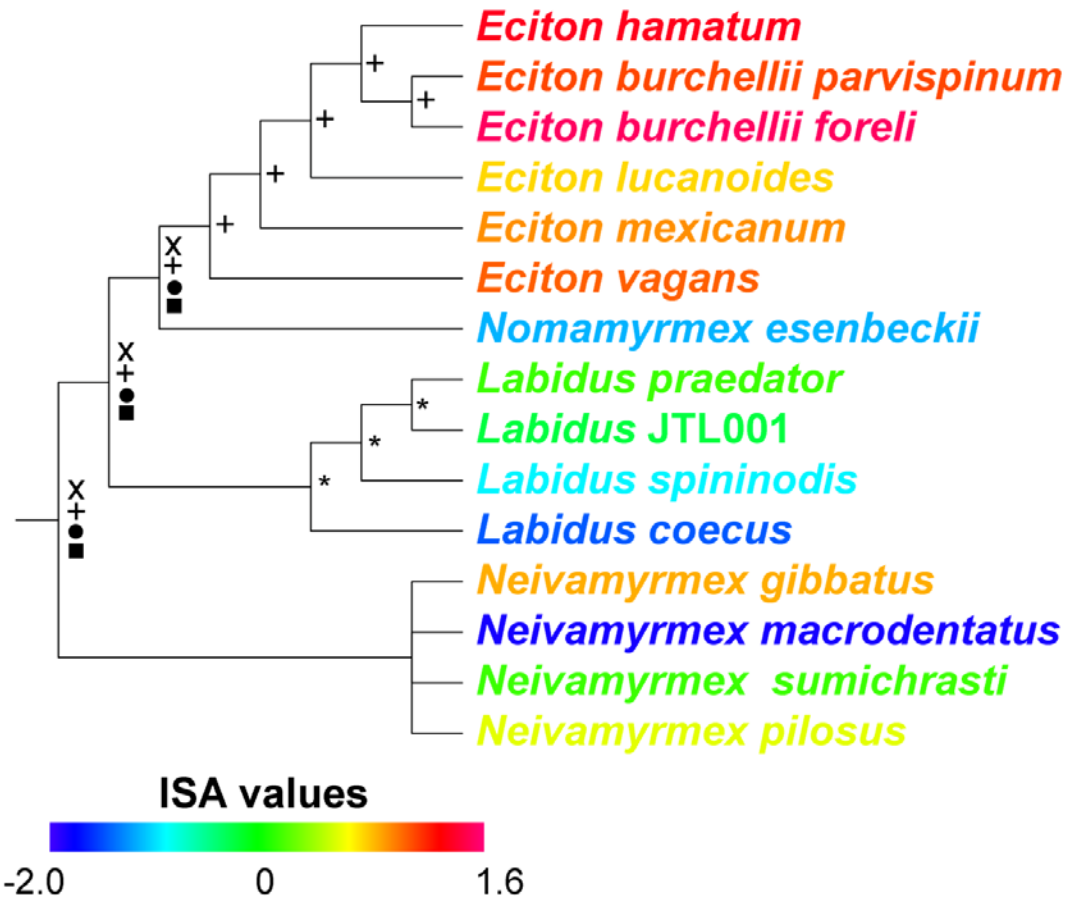

Figure 3-2. Phylogeny of 15 Doryline species sampled, used for calculating independent contrasts; citations for each node are noted: + Winston, Kronauer and Moreau (2017), * Barth, Moritz and Kraus (2015), ● Brady *et al.* (2014), ■ Brady (2003), x Borowiec (2016); color corresponds to index of surface activity with cool colors representing more subterranean species

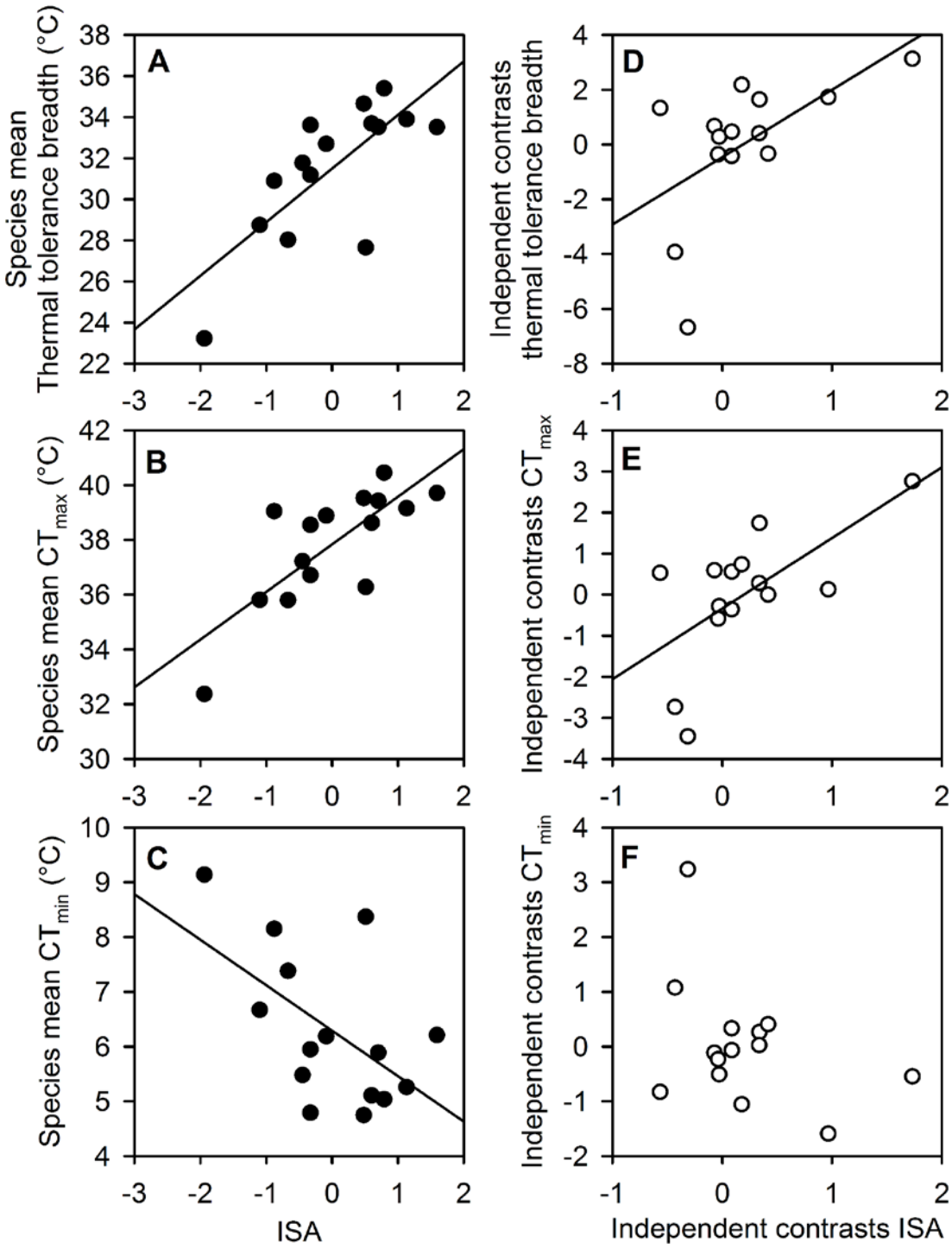

Figure 3-3. Correlation between species index of surface activity (ISA) and species means for three thermal tolerance metrics; filled points represent raw data, open points represent phylogenetic independent contrasts which control for relatedness

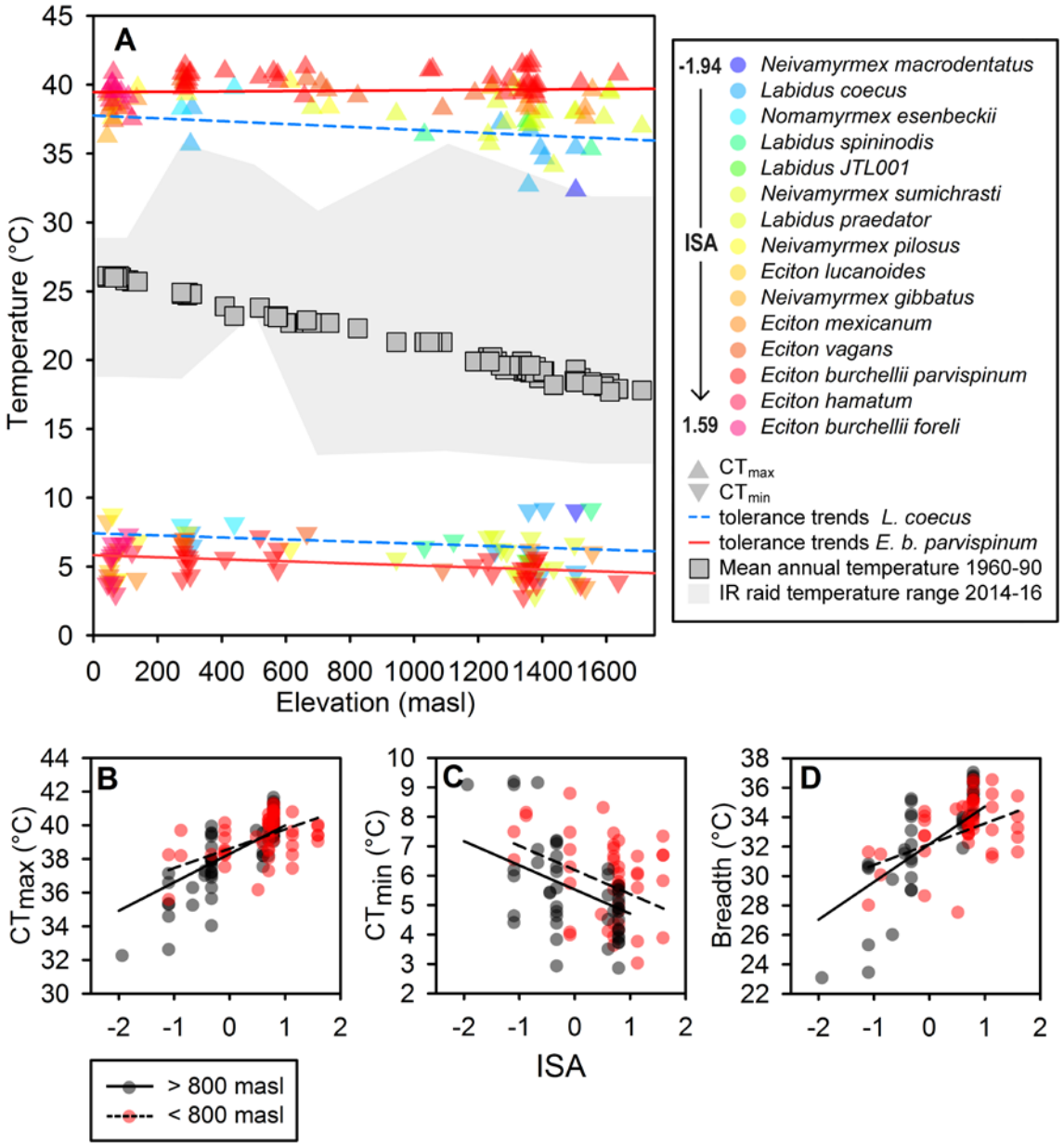

Figure 3-4. Effect of elevation and species microhabitat use on thermal tolerance; all points represent colony mean values (A) upwards pointing triangles are colony average CT_{max} and downward pointing triangles are colony average CT_{min}; Assuming a head width of 1.28 mm red dotted lines are trends for thermal tolerance in *E. burchellii parvispinum* (ISA= 0.79) and the blue dashed line is the trend for *L. coecus* (ISA= -1.10); slopes did not significantly differ among ISA groups for CT_{min}; grey shaded area is the range of surface raid temperatures recorded at each site, grey squares are bioclim extrapolations for mean annual temperature, extracted using raid coordinates (B) ISA versus CT_{max} at high and low elevations (C) ISA versus CT_{min} at high and low elevations (D) ISA versus thermal tolerance breadth at high and low elevations

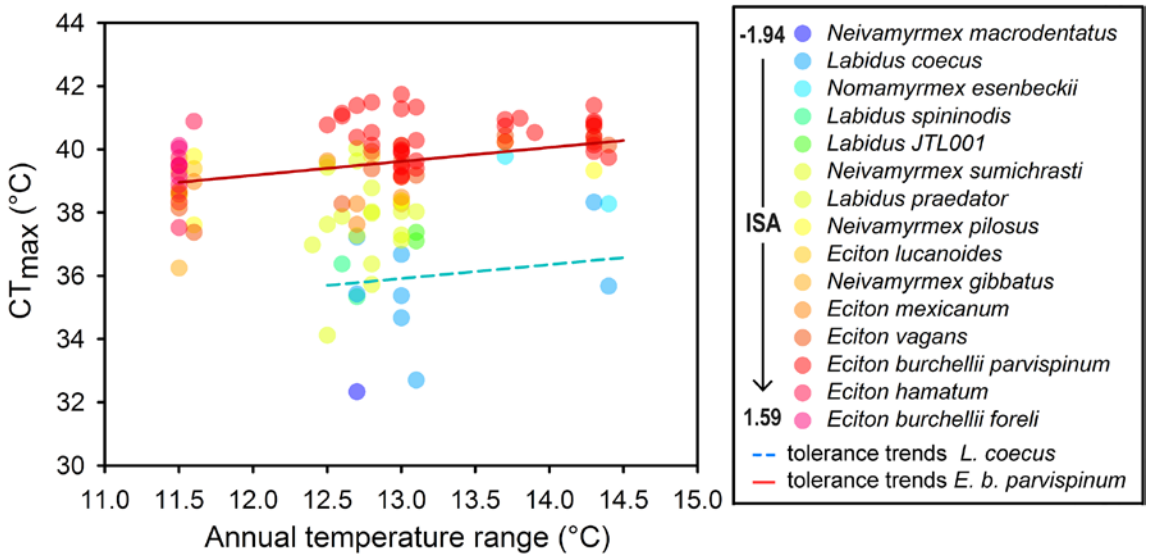

Figure 3-5. The effect of annual range in temperature on CT_{max}; warm color points represent more aboveground (higher ISA value) species; the red solid line represents the estimated trend of an *E. burchellii parvispinum* worker with head width 1.28 mm; the blue dotted line represents is the estimated trend of an *L. coecus* worker with head width 0.8mm

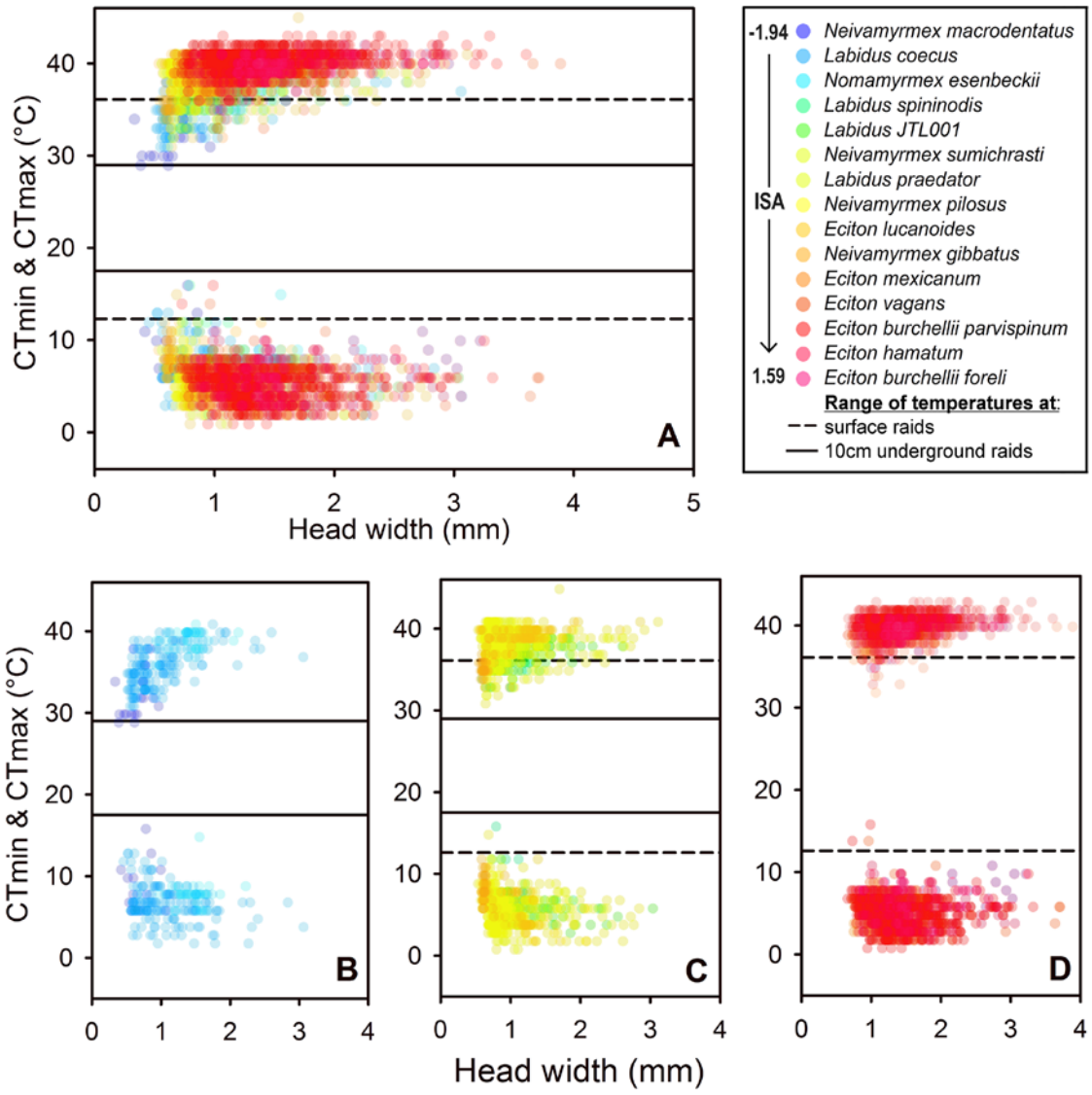

Figure 3-6. (A) Effect of body size on individual thermal tolerance; legend depicts species in order of increasing surface activity index (ISA), warmer colors are more surface-active species, cooler colors are more subterranean species, solid lines are maximum and minimum underground raid temperatures, dotted lines are maximum and minimum surface raid temperatures; (B) Effect of body size on individual thermal tolerance for *N. macrodentatus*, *L. coecus*, and *N. esenbeckii*; (C) Effect of body size on individual thermal tolerance for *L. spininodis*, *L. JTL001*, *L. praedator*, *N. pilosus*, *E. lucanoides*, and *N. gibbatus*, (D) Effect of body size on individual thermal tolerance in *E. mexicanum*, *E. vagans*, *E. burchellii parvispinum* , *E. hamatum*, *E. burchellii foreli*

CHAPTER 4: STRUCTURE AND THERMAL BIOLOGY OF SUBTERRANEAN ARMY ANT BIVOUACS IN TROPICAL MONTANE FORESTS (*Labidus praedator*)

Previously peer reviewed and published journal article (Baudier & O'Donnell 2016).

Introduction

The typically soft-bodied, altricial brood of many social insects are more sensitive to thermal variation than adult nest mates (Nalepa 2011). Social insect nests are often thermally homeostatic with temperature control achieved by passive and/or active thermoregulation (Seeley & Heinrich 1981; Jones & Oldroyd 2007). Passive thermoregulation involves behavioral responses to environmental thermal gradients (Jones & Oldroyd 2007). Examples of passive thermoregulation among social insects include foraging site and nest site selection, nest construction and orientation, and relocation of brood within nests (Coenen-Stass, Schaarschmidt & Lamprecht 1980; Frouz 2000; Penick & Tschinkel 2008). Actively thermoregulating organisms use physiology to modify internal or ambient temperatures via metabolic heating or physical activity (Seeley & Heinrich 1981; Jones & Oldroyd 2007). Examples of active thermoregulation among social insects include worker clustering, flight muscle twitching to generate heat, and wing-fanning to promote evaporative cooling (Heinrich 1993; Anderson, Theraulaz & Deneubourg 2002; Weiner *et al.* 2010). Most ant species rely on passive thermoregulation to modify nest temperatures because workers are wingless, preventing active thermoregulation via fanning or flight muscle shivering (Hölldobler & Wilson 1990). However, some Neotropical army ants in the subfamily Dorylinae create homeostatic thermal conditions within their temporary nests (bivouacs) via active thermoregulation (Jones & Oldroyd 2007). In two above-ground active species, *Eciton burchellii* (Westwood) and *Eciton hamatum* (Fabricius), bivouacs are composed of hundreds of thousands of clustering worker bodies that surround and insulate the brood and queen. These bivouacs are actively warmed via collective metabolic heat, and bivouac temperature variation is lower than ambient (Schneirla, Brown & Brown 1954; Jackson 1957;

Coenen-Stass, Schaarschmidt & Lamprecht 1980; Franks 1989; Anderson, Theraulaz & Deneubourg 2002; Jones & Oldroyd 2007; Kadochová & Frouz 2014). All other army ant species bivouac below ground (Rettenmeyer 1963; Berghoff *et al.* 2002). Here, I present the first measurements of subterranean bivouac thermal physiology and brood developmental synchrony in the army ant *Labidus praedator* (Smith 1858).

Previous studies of army ant thermoregulation have primarily focused on above-ground *E. burchellii* and *E. hamatum* bivouacs (Schneirla, Brown & Brown 1954; Jackson 1957; Rettenmeyer 1963). Lowland *E. burchellii* bivouacs thermoregulated their brood at relatively stable elevated temperatures of 28 ± 1°C (average 2°C higher than ambient, maximum 6°C higher than nocturnal low temperatures) (Schneirla, Brown & Brown 1954; Franks 1989), while lowland bivouacs of *E. hamatum* were on average 1°C higher and less thermally variable than ambient conditions of 22–29°C (Jackson 1957). Active bivouac thermoregulation and homeostasis is thought to be tightly linked to the synchronous brood development cycles that characterize the dichotomous statary (egg/pupal) and nomadic (larval) phases of *Eciton* colony activity (Schneirla, Brown & Brown 1954; Jackson 1957; Franks 1989). Whether active or passive bivouac thermoregulation and brood synchrony occur in other species of Dorylinae is unknown. *Eciton* species are not representative of Dorylinae, in part because they raid and frequently bivouac above ground (Schneirla 1933; Schneirla, Brown & Brown 1954; Rettenmeyer 1963; Gotwald Jr 1995). In contrast, most Neotropical army ant species are at least partly subterranean, bivouacking underground and raiding partly or entirely below ground (Rettenmeyer 1963; Gotwald Jr 1995). These differences in foraging and bivouacking soil microhabitat correspond to differences in sensory investment and thermal tolerance (Baudier *et al.* 2015; Bulova *et al.* 2016). Due to the challenge of tracking underground mobile nests, the subterranean bivouacs of many common army ants remain undescribed (Rettenmeyer 1963; Berghoff *et al.* 2002; Dunn 2003). Above-ground bivouacking in *Eciton* is a derived state among army ants (Brady 2003; Brady *et al.*

2014). Therefore, data on bivouacking behavior in other doryline genera can provide evidence of how bivouac thermoregulation evolved in the above-ground species.

Labidus praedator is an abundant subterranean bivouacking army ant that raids both on the surface of the forest floor and underground (Rettenmeyer 1963; Kaspari & O'Donnell 2003; O'Donnell *et al.* 2007). *Labidus praedator* ranges from the southern United States (N 30°50', W 93°45') to Argentina and Southern Brazil (S 30°20', W 51°12'). *Labidus praedator* occurs in the lowlands near sea level, though this species is most abundant from 1000 to 1600 m asl in Costa Rica (Schneirla 1949; Watkins 1976; Kaspari & O'Donnell 2003; Longino 2010; O'Donnell *et al.* 2011; Economo & Guénard 2016). Brood development in *L. praedator* is apparently synchronous with statary and nomadic colony phases observed in Neotropical lowland wet forests of Panama (N 09°09', W 79°51') as well as in the tropical dry forest of southern Mexico (N 18°27', W 96°12') (Schneirla 1947; Rettenmeyer 1963; Schneirla 1971). Possible evidence of seasonal asynchrony in brood development has been reported at the southernmost extent of this species' range in Paraguay (S 25°20', W 57°32') during extended winter statary phases with air temperatures reaching below 12.5°C at night (Fowler 1979). Fowler (1979) suggested temperatures within a Paraguay *L. praedator* bivouac were less variable than surface air temperatures but bivouac temperatures were not elevated. However, Fowler (1979) measured temperature at a single bivouac point and did not measure adjacent soil temperatures, making interpretation of the data problematic. I asked whether tropical *L. praedator* colonies warm and/or buffer temperatures within their bivouacs when exposed to relatively low ambient temperatures in montane and premontane forest.

Air temperatures are relatively low year-round in high elevation tropical sites, which can select for localized lowtemperature adaptations (Janzen 1967; Ghalambor *et al.* 2006). However, army ant colonies are nomadic. For example, colonies of the highly epigaeic army ant *Eciton burchellii parvispinum* (Forel) move nomadically across elevations in premontane and montane forests of Monteverde, potentially experiencing a variety of mean annual temperatures at different

elevations (Soare *et al.* 2014). *Eciton burchellii* bivouacs are more likely to be located in sheltered refuges in montane forest than in lowland forest, suggesting army ant nest site selection is a behavioral mechanism for dealing with thermal challenges (Soare *et al.* 2011). Underground environments have reduced daily and seasonal thermal variation, but closely match the local annual mean air temperature (Harkness & Wehner 1977; Parton & Logan 1981; Tschinkel 1987). Subterranean bivouacking *L. praedator* therefore likely experiences less temporally variable temperatures, but similar geographic (elevational) variation in mean temperature, compared to surface bivouacking army ants. To date, there are no published records of bivouacking behavior or thermal biology for montane or premontane subterranean army ants. Here, I report observations of the structure and thermal properties of subterranean bivouacs of *L. praedator* from montane forests (1500–1565 m asl) and premontane forest (950 m asl) near the center of this species' latitudinal range in Costa Rica (approximately 10°N) (Watkins 1976). I compared bivouac temperature conditions with thermal tolerances of the ants and their symbionts, and addressed two questions regarding bivouac thermal properties: 1. do subterranean army ants maintain elevated bivouac temperatures? 2. Do surface or sub-surface conditions exceed thermal tolerance limits of army ant workers or symbionts at high elevations, and do bivouacs buffer against these conditions?

In tropical premontane wet forests of Monteverde, Costa Rica, mean annual temperature at 1460 m asl is 18.8°C (Nadkarni & Wheelwright 2000). I recorded average air temperatures of 15.9°C while recording bivouac temperatures at 1565 m asl in March of 2015; March is a relatively cool dry-season month in Monteverde (Nadkarni & Wheelwright 2000). This temperature is lower than optimum for brood development in most tropical ant species (Franks 1989; Abril, Oliveras & Gómez 2010; Kipyatkov & Lopatina 2015). I therefore predicted active metabolic warming would be used to elevate *L. praedator* bivouac temperatures at this high elevation site.

Army ant colony members may not be the sole beneficiaries of a climatically moderated bivouac. Army ant colonies are host to the most species-rich array of animal associates known to science (Rettenmeyer *et al.* 2011). Many of these nest associates are arthropod species that live within the bivouac (Rettenmeyer 1962b; Eickwort 1990; Beeren, Maruyama & Kronauer 2016; Parker 2016). Myremecophiles that live within the nest of their hosts are referred to as inquilines (Rettenmeyer 1962a). Little is known about potential thermal benefits of inquilinism, but the thermal biology of army ant bivouacs is potentially relevant to the climate niche and responses of these ants and of associated symbionts to climate change. Brood, callow (newly eclosed) workers, and myrmecophilic inquilines are seldom seen outside bivouacs except during colony emigrations, suggesting bivouacs could buffer them from thermal extremes (Schneirla, Brown & Brown 1954; Rettenmeyer 1962a; Rettenmeyer 1963; Rettenmeyer *et al.* 2011). I tested whether surface or sub-surface conditions exceeded the thermal tolerance limits of inquiline Calymmodesmus sp. millipedes collected from a subject *L. praedator* bivouac.

Methods

Nest structure

Two *L. praedator* bivouacs were observed in montane forest in July 2014, March 2015, and June 2015 on the Pacific slope of the continental divide near Monteverde, Costa Rica. Another bivouac was observed in premontane forest in April 2016 in the Children's Eternal Rainforest near San Gerardo Research Station on the Atlantic slope. The taxon referred to here as *L. praedator* was morphologically consistent with 'matte-face' Labidus sp. Cac 1 as described by Barth, Moritz and Kraus (2015). External nest structure and ant activity were observed over multiple days for two active bivouacs (bivouacs A and B) as follows:

Bivouac A (N10°18.113' W84°48.109', 1500 m asl) was observed active 11–22 July 2014. During this time I noted external nest structure, lightly probing the bivouac with a machete

and spade to assess ant presence and activity in the vicinity of the surface. I encountered bivouac A on 11 July 2014, and checked the bivouac site four additional times on 20 July 2014, 22 July 2014, 27 July 2014, and nearly 1 year later on 18 June 2015 (after the ants had departed). The ants had emigrated sometime between 22 July and 27 July 2014, the last two observations being of the evacuated bivouac site. I did not excavate this bivouac to observe brood developmental stage. However, the absence of discarded pupal cases prior to and after the colony's emigration from this site suggests the colony's brood were larvae. Larval brood are associated with the nomadic phase in *Eciton* (Schneirla, Brown & Brown 1954; Jackson 1957).

Bivouac B (N10°17.816' W84°47.951', 1565 m asl) was an active statary-phase bivouac observed 19–26 March 2015. Observations of surface nest structure, colony presence and surface activity were made during this time in the same manner as for bivouac A. Bivouac B was first seen on 19 March 2015. I checked the bivouac site seven additional times on 20, 21, 22, 24, 25 and 26 March 2015, and 18 June 2015. my March field season ended before the emigration of this colony, but the ants were absent from this site on 18 June 2015. After 5 days of temperature data collection (below), bivouac B was excavated twice, followed by observations and photographing of internal structure. The two excavations of bivouac B took place 24 h apart (25 and 26 March 2015), and yielded consistent results, the ants having reformed the bivouac structure overnight.

Bivouac C (N10°22.375' W84°46.532', 950 m asl) was an active statary-phase bivouac observed on 22 April 2016. Due to a mat of thick buttress roots associated with the bivouac, a full excavation could not be completed, but I performed a partial excavation and nest surface description.

Temperature and humidity measurements

Temperature and relative humidity of bivouac B were recorded using alternating ibutton hygrochron (measuring humidity and temperature), and thermochron (measuring only temperature) data loggers placed alternately every 10 cm along a thermally inert (wooden)

vertical probe from soil surface to 40 cm depth in the bivouac (ibutton: Maxim IntegratedTM, San Jose, CA, USA) (Figure 4-1). A reference probe with an identical configuration was placed in soil 1 m away from the bivouac (Figure 4-1). Data were collected every 5 min for 5 days. Temperature accuracy of all ibuttons was confirmed to be within ±0.5°C (the manufacturer-reported instrument error for thermochrons) via hot and cold water bath (42 and 0°C respectively) using a certified glass thermometer. Relative humidity accuracy of hygrochrons was confirmed to be within ±1% at 0 and 100 % by using desiccants and suspension over enclosed water bath at 25°C. Additional temperature readings were taken at various depths during excavation using a hand-held infrared (IR) thermometer (BAFX Products, Milwaukee, WI, USA). IR thermometer accuracy was confirmed using a calibrated thermocouple. Temperatures were recorded with the IR thermometer for the bivouac C surface, soil surface 1 m away from the bivouac, and in the upper portions of the underground bivouac cavities at 10 and 15 cm (within a gallery underneath one of the roots).

Thermal tolerance

Critical thermal maximum (CT_{max}) and minimum (CT_{min}) were measured for callow and non-callow workers of different body sizes, as well as for inquiline millipedes found within bivouac B. CT_{max} and CT_{min} were measured using standard dynamic methods (Lutterschmidt & Hutchison 1997; Diamond *et al.* 2012; Oberg, Toro & Pelini 2012) with thermal ramping at a rate of 1 °C every 10 min. Insects showing immobility for a duration of 10 s were considered to have surpassed their CT_{max} or CT_{min} respectively. Half of the ants were allowed to acclimate to lab conditions for 24 h prior to thermal assays (ranging from 19 to 26 °C over the course of the day), while half were run in thermal assays 30 min after collection. The thermal tolerances of these groups were compared to test for effects of acclimation.

Statistical analyses

All analyses were performed in R statistical software. I used standard linear multifactor analyses of variance to identify significant predictors of bivouac temperatures, environmental temperatures and thermal tolerances (Quinn & Keough 2002). I used the ANOVA function to test for significance of predictors by comparing the fit of linear models with and without the inclusion of each predictor variable. Starting with all measured predictors, Aikake Information Criteria (via the drop1 function) was used to select the order of predictor variable testing and elimination from the model. If the predictor had a significant effect on the model's fit, it was included in the full model for subsequent analyses. Mean, maximum and minimum daily temperatures and relative humidity were compared across depths and treatments (bivouac versus soil reference probes). Linear regressions of soil depth versus daily mean were also performed within each location. Predictors of CT_{max} and CT_{min} included head width (as a proxy for body size), whether the ant was from the acclimated or non-acclimated group, and whether ants were callow. Student's t tests were run to compare millipedes to all ant size classes for both CT_{max} and CT_{min}.

Results

Nest surface structure

Bivouac A was found active at the base of saplings and a small tree trunk at 1500 m asl on 11 July 2014 (Fig. 2, Supplementary Figure 4-1). Bivouac B was found alongside and below the root mass of a fallen tree on 19 March 2015 at 1565 m asl (Figure 4-1, Supplementary Figure S4-1). Bivouac C was found between and beneath the intertwining roots of two live trees at the top of a ridge on 22 April 2016 at 950 m asl (Supplementary Figure 4-1). The surface structure of all three bivouacs consisted of low, wide mounds of loose excavated soil (in fine particles) intermixed with colony refuse. In all cases, the excavated soil was distinctive and easily visible against nearby leaf litter.

The surface mound of bivouac A was approximately circular and larger in area than the other two mounds(length: 124 cm, width: 141 cm). The surface mound of bivouac B was crescent-shaped around the base of the root mass of a fallen tree (length: 59 cm, width: 32 cm). Mid-elevation bivouac C was visible on the surface as a series of small mounds between root buttresses of two mature trees (entire area length: 81 cm, width: 76 cm). Unlike the other two observed bivouac mound surfaces, that of bivouac B was punctuated with craters approximately 1 cm in diameter. All three bivouacs were covered in a mixture of excavated soil and colony refuse. For bivouac B, this refuse consisted largely of discarded pupal cases, isopod tergites and other arthropod body parts (Supplementary Fig. 2). The surface of bivouac C was covered in what appeared to be fine bits of excavated decomposing wood in addition to excavated soil and colony refuse (cockroach tergites, isopod tergites, and discarded ant pupal cases).

The lack of discarded pupal cases on the bivouac surface of bivouac A suggests the colony was nomadic at the time. I also noted two possible abandoned bivouac sites within a 500 m radius of bivouac A with similar loose soil and broad circular shape, also located at the base of saplings. Bivouac B was in the late statary phase, with the majority of the pupae having eclosed by the time my observations in March 2015 were complete. Bivouac C was likely in the late statary phase as well, having large numbers of discarded army ant pupal cases among colony refuse.

In June of 2015, former bivouac sites of bivouac A and bivouac B were revisited and found to be void of any army ant activity. At this time, the soil on the surface of both abandoned nest mounds appeared to have sunken in the absence of *L. praedator* (27 cm subsidence for bivouac A, 17 cm subsidence for bivouac B) (Supplementary Figure 4-3).

Internal nest structure and colony strata

The initial excavation of bivouac B was performed at 5 pm. At that time, some ant activity was observed on the soil surface. Excavating the top 10 cm of soil produced considerable defensive

activity with the arrival of several hundred soldiers (also observed in bivouac A the previous year and bivouac C the following year). Upon excavation, bivouac B contained mature workers, callow workers of various size castes and hundreds of pupae, indicating synchronous brood development and a colony in the late statary phase; no army ant larvae were observed. The depth at which the first callow workers were observed was 17 cm, however, callow workers were at highest density from depths of 20–35 cm. These callow workers were interspersed with pupae found from 27 to 35 cm depth (Figure 4-1).

Bivouac B did not appear to occupy a large central cavity, but rather consisted of many small tunnels (<1 cm) and chambers within loose soil. Soil adjacent to the bivouac was more compacted and less porous than soil within the bivouac, particularly around roots of the fallen tree. Some tunnels were close to small (0–1 cm diameter) roots of this fallen tree, though the majority of the bivouac structure consisted of small interconnected chambers and tunnels independent of the tree's root system. On the second day of excavation, bivouac structure was similar, the bivouac having been reconstructed over night. There was one exception: fewer pupae were present than the previous day and callow workers were relatively more abundant. This is likely due to overnight eclosion of workers. I did not observe the emigration of bivouac B.

Excavating bivouac C within 10 cm of the surface of the bivouac revealed large galleries (approximately 2 cm in diameter) beneath some of the most superficial, large (>10 cm diameter) roots.

Nest-associated arthropods

Numerous white inquiline millipedes (genus *Calymmodesmus*) were found within bivouac B (Loomis 1959; Rettenmeyer 1962b). Calymmodesmus were in high density in the vicinity of the pupae and extended from 20 cm to 43 cm depth in the bivouac (Figures 4-1, 4-3). Other inquilines included one other species of millipede, two morpho-species of Acari, two morpho-species of wingless phorid found roaming the mound surface, and five species in family

Staphylinidae (including one species in subfamily Scydmaeninae) found roaming atop the refuse-covered bivoac. Other notable associates encountered include one species of winged Phoridae that arrived en masse when I excavated high ant-density portions of the nest, as well as two morpho-species of Collebmola (1sp. Entomobryidae, 1sp. Poduroomorpha). Only *Calymmodesmus* millipedes were used in thermal tolerance assays as they were the only myrmecophile collected in sufficient numbers to enable within-species replication of critical thermal measures. Photographs of each collected myrmecophile morpho-species are included in supplementary materials (Supplemental Figures S4-4 – S4-16).

Temperature and humidity

Temperatures at bivouac B as measured by IR thermometer during excavation were 14–15°C on the surface of the soil 1 m away from the bivouac, with 23.1°C measured at the brood center of the bivouac during excavation. This was slightly higher than the maximum temperature of 22.6°C recorded by the 40 cm depth ibutton probe in the bivouac. Worker ants may have moved among bivouac depths in response to variation in surface temperature. At 7:15 am on a cold morning (13.6°C measured by ibutton on bivouac surface), adult worker ants were not observed at depths less than 13 cm, while on a warm day (surface ibutton measured 19.1°C at 9:49 am) ant activity was observed within 1 cm of the surface of the bivouac.

Mean, maximum and minimum daily temperatures (recorded by ibuttons) were always equal or higher in the bivouac than at depth-matched reference points in the soil nearby (Figures. 4-4, 4-5; mean $F_{1,46} = 81.75$ $p<0.001$; maximum $F_{1,46} = 166.55$ $p<0.001$; minimum $F_{1,46} = 59.09$ $p<0.001$). Mean daily temperature increased with depth from the surface within the bivouac ($R^2 = 0.91$, $F_{1,23} = 245$, $p<0.001$); the mean temperature at 40 cm depth was 6.2°C higher than the depth-matched reference point in soil (Figure 4-4). Mean temperatures (±standard deviation) experienced by pupae at depths of 30–40 cm were 21.8±0.4°C. Daily mean belowground temperatures were only slightly higher than surface (0.8°C at 40 cm depth) in soil with no ants

(R^2 = 0.75 $F_{1,23}$ = 72, p<0.001) (Fig. 4-4). Time of day was a significant predictor of temperature ($F_{1,286}$ = 212.46, p<0.001), accounting for much of surface thermal, 10 cm, and 20 cm variability in both the bivouac and depth-matched reference points (Figure 4-5). Daily fluctuation in temperature decreased similarly with soil depth in both the reference and bivouac samples (Figure 4-5). Standard deviation of bivouac temperatures at each depth was equal to or slightly greater than those in the nearby soil (Figure 4-4). The bivouac therefore experienced reduced variation in temperature relative to surface air temperature, but did not experience reduced variation in temperature compared to nearby soil. Relative humidity remained near 100 % as recorded across all surface and sub-surface probes for both bivouac and soil reference transects (Figure 4-4). Daily maximum humidity was 100 % for all depths regardless of ant presence. Minimum relative humidity across 5 days was 88.1 % on the surface with a minimum bivouac humidity of 96.7 %.

Ambient and bivouac surface temperatures at premontane bivouac C were higher (as measured using IR thermometer) than corresponding temperatures at montane bivouac B. At 13:52 on 22 April 2016, surface soil temperatures 1 m from bivouac C ranged from 21.6 to 22.0°C, while the surface of the bivouac was 22.0–23.6°C. 10 cm below the bivouac surface ranged in temperature from 24.0 to 24.8°C, and within a gallery beneath a root approximately 15 cm below the surface, temperatures ranged from 24.5 to 25.3°C.

Thermal tolerances

CT_{max} (F1,10 = 0.001, p = 0.919) and CTmin (F1,11 = 0.011, p = 0.973) did not differ with 24 h of acclimation. Acclimation treatments were therefore pooled in subsequent analyses. Larger ants had higher CT_{max} (F = 12.53, df = 14, p = 0.004), but CT_{min} did not covary with body size (F1,14 = 2.540, p = 0.135). Callow ants had higher CT_{min} than non-callow ants (F1,13 = 106.66 p\0.001), while CT_{max} did not differ significantly between callow and non-callow ants (F1,12 = 0.489 p = 0.498; Figure 4-4). CT_{max} was higher for mature worker ants than inquiline millipedes (F = 19.119, p\0.001). *L. praedator* workers also had lower CT_{min} than millipedes (F = 28.619,

p\0.001). Low temperatures were not below CT_{min} for any of the assayed individuals at the bivouac depths where they occurred, (Figure 4-3). However, minimum recorded surface temperatures were colder than tolerable by the most sensitive millipedes, and were only 1.1 °C warmer than tolerated by the most sensitive callow workers (Figure 4-3; Supplementary Table S4-1).

Discussion

Bivouac site selection

All observed active and apparently abandoned montane and premontane *L. praedator* bivouac sites occurred at the base of live or dead trees (Supplementary Figure S4-1), suggesting (together with previous accounts) that *L. praedator* selects bivouac sites in loose soil or cavities created by live tree roots, fallen trees, or other similar structures (Sumichrast & Norton 1868; Rettenmeyer 1963; Sudd, Schneirla & Topoff 1972; Monteiro, Sujii & Morais 2008). However, both high volumes of loose soil on the surface and soil subsidence after ant departure indicate excavation by the ants (Supplemental Figure S4-2). Bivouac B was able to rebuild overnight suggesting rapid excavation of several liters of soil.

Bivouac thermal and humidity conditions

Above-ground bivouacking army ants achieve nest homeostasis via active metabolic warming and active thermal buffering using the interlocked bodies of the ants themselves (Schneirla, Brown & Brown 1954; Franks 1989; Jones & Oldroyd 2007). my data show that two high elevation *L. praedator* colonies at a tropical latitude (N10°18’) actively warmed their bivouacs via collective metabolic heating, while possibly relying on passive thermal buffering effects of soil to reduce dial thermal fluctuations in temperature. Nest heating via combined metabolic activity of ant workers in close spatial proximity is an active thermoregulation mechanism (Jones

& Oldroyd 2007), while bivouac moderation of widely varying air temperatures could not be distinguished from passive soil buffering. The *L. praedator* placement of immobile pupae and callow workers within bivouac B along the thermal gradient, and adult worker movements in response to solar warming, are passive thermoregulatory mechanisms common among many ant species (Jones & Oldroyd 2007; Penick & Tschinkel 2008).

The warmest regions of the statary *L. praedator* bivouac B were the lower level strata containing the brood (pupae) and millipedes. Thermal probes showed these regions were on average 21.8 ± 0.4°C, with infrared recordings of up to 23.1°C at the brood center. This is not as high as previously suggested thermoregulatory target windows of E. *burchellii* (28 ± 1°C) and *E. hamatum* (26.6 ± 1.1°C) in the lowlands (Jackson 1957; Franks 1989). At 40 cm depth from the surface, the *L. praedator* bivouac sustained mean daily temperatures 6.2°C higher than at the same depths in surrounding soil. This is a greater and longer-sustained warming effect than previously reported for any army ant bivouac (Jackson 1957; Franks 1989).

I also recorded higher bivouac surface temperatures relative to soil surface temperatures 1 m away, and increasing temperatures with bivouac depth, within the superficial portions of mid-elevation bivouac C (950 m asl). Both suggest that bivouac metabolic warming is not a montane bivouac phenomenon. However, the fact that even superficial portions of mid-elevation bivouac C were warmer than the warmest portions of montane bivouac B (1550 m asl), suggests that internal bivouac temperature is not uniform across elevations, and that high elevation bivouacs may struggle to raise bivouac temperatures to those optimum for brood. Temperatures in above-ground bivouacs at high elevations have yet to be measured, but will likely shed further light on this interplay between microhabitat and elevational thermal effects on bivouac warming.

Ants in general, and particularly soft-bodied ant larvae, are susceptible to desiccation in a wide variety of environments (Hölldobler & Wilson 1990). For bivouac B, although my humidity and temperature measurements were taken during the dry season, sub-surface and surface relative humidities near 100 % were recorded regardless of ant presence (Figure 4-4). This suggests *L.*

praedator bivouacs are not limited by moisture availability in the lower montane wet forest life zone. Future studies of how *L. praedator* bivouacs respond to dry season conditions in seasonally dry forests (such as in Guanacaste) may shed light on adaptations for dealing with low humidity.

The presence of discarded tergites in the refuse of *L. praedator* confirms previous observations that this species feeds largely on isopods in the premontane life zones of Monteverde (Supplementary Figure S4-2) (Longino, J. T. pers. comm.) (Longino 2010), with evidence of small cockroaches and other insects consumed as well. I observed *L. praedator* feeding on terrestrial amphipods at a raid fronts in Monteverde and San Gerardo in June 2015 and April 2016 respectively. Although I observed refuse atop all montane and premontane bivouacs, there are no accounts of either loose-dirt mound construction or refuse-topping in low elevation *L. praedator* bivouacs (Sumichrast & Norton 1868; Rettenmeyer 1963; Fowler 1979). Whether this refuse topping serves as an adaptive thermal warming function remains to be tested.

Thermal sensitivities of bivouac occupants

Inquiline millipedes (Calymmodesmus sp.) were more thermally sensitive to both heat and cold than mature or callow ants, while callow worker ants differed from mature workers in their ability to function at low temperatures. The higher CTmin of callow workers may be related to differences in cuticular thermal resistance (Galushko *et al.* 2005). The impact of cold on callow movement may, at least in part, explain the common observation of callow workers being carried in emigrations immediately following the statary phase (Rettenmeyer 1962a; Rettenmeyer 1963). In the case of Calymmodesmus millipedes, bivouac surface temperatures were within 1.1 ° C of the mean CTmin for all millipedes, and were lower than what could be tolerated by the most sensitive individuals. Choice of location within the bivouac corresponded to these sensitivities, with Calymmodesmus being found deep within the nest where temperatures were farthest from their CTmin. These findings show millipede thermal specialization to bivouac homeostatic conditions, suggesting an obligate relationship between host and inquiline. This more thermally

stable region of the bivouac was also where the pupae were housed, indicating a narrow thermal tolerance range for *L. praedator* pupae. Callow workers were less sensitive than millipedes and correspondingly were encountered at shallower depths than inquiline millipedes or pupae, where temperatures are more variable over the course of the day. The widest distribution of activity within the bivouac was seen by the mature workers, inhabiting bivouac depths from 0 cm down beyond 45 cm in low densities. The cold sensitivity of callow adult workers combined with low temperature ambient conditions suggests that active warming in *L. praedator* bivouacs is an adaptive response to cold limitation at this high elevation site.

CHAPTER 4: FIGURES

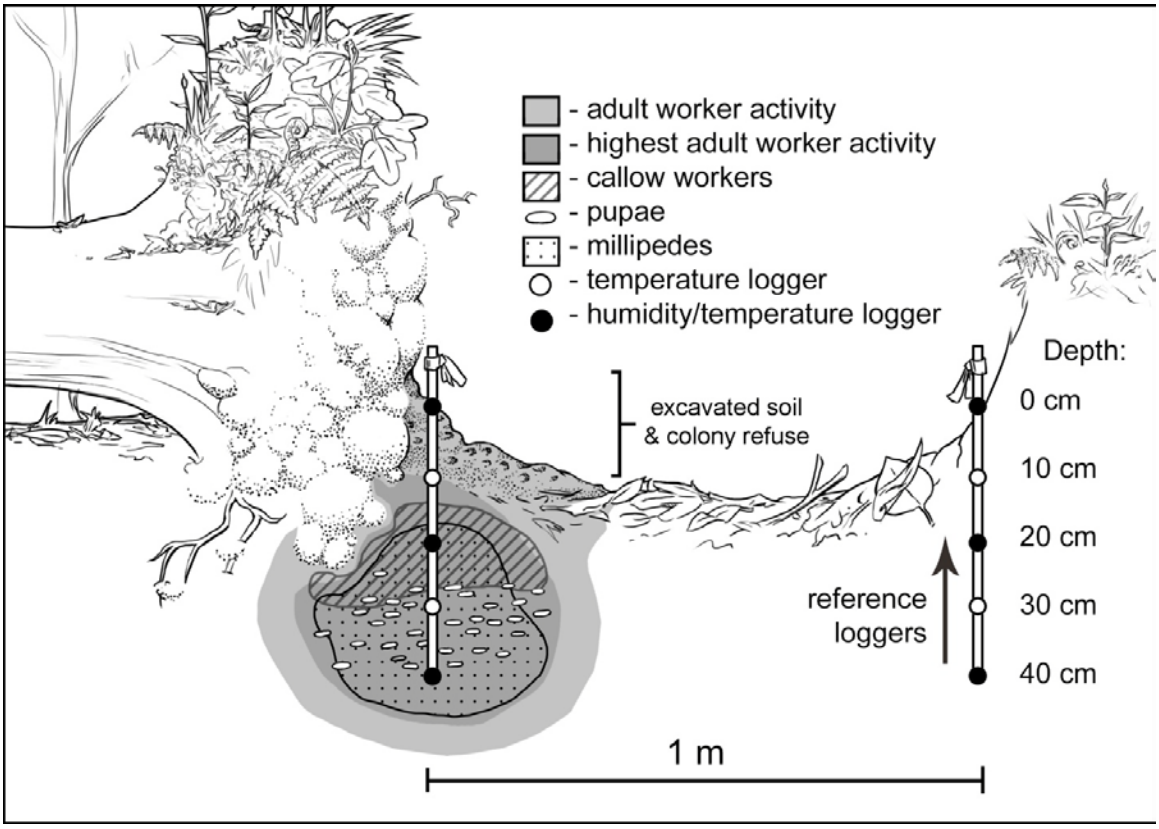

Figure 4-1. Structure and probe placement for temperature and humidity measurements of bivouac B

Figure 4-2. Surface nest structure of bivouac A on 11 July 2014, showing the loose soil mound covered in fine bits of colony refuse; cleared bivouac surface appeared prominent and easily distinguished from surrounding leaf litter

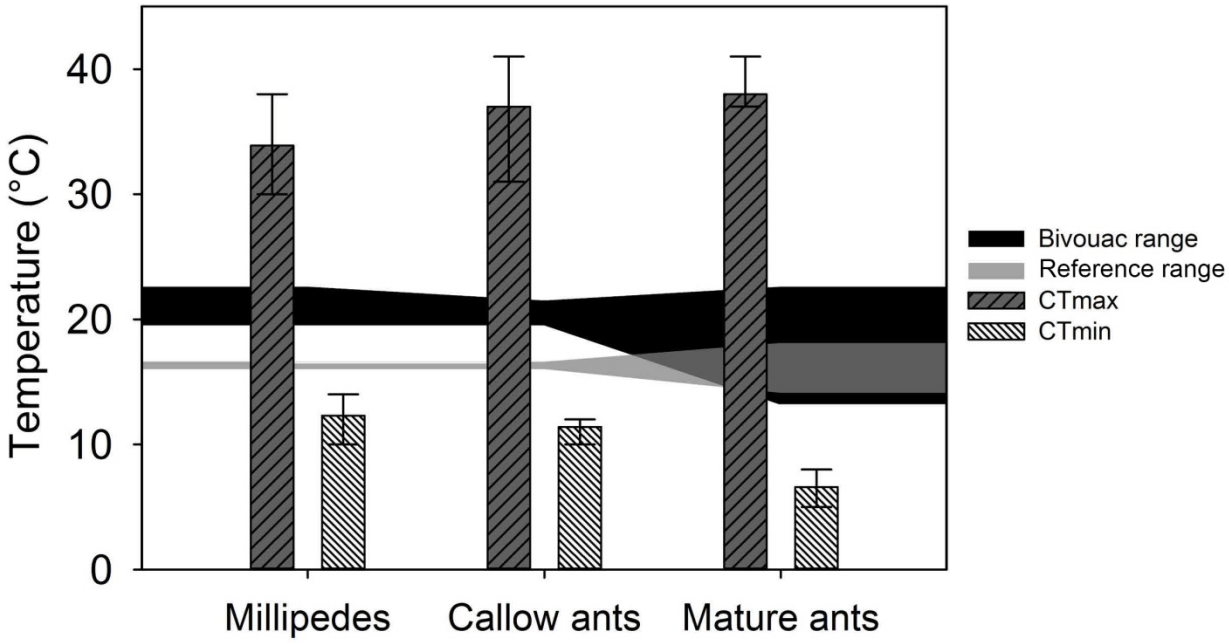

Figure 4-3. Thermal tolerances of three types of assayed subjects extracted from the bivouac; black backround area represents the bivouac temperatures recorded within the range of depths where each category was collected; grey backround area represents reference probe temperatures at equivalent depths

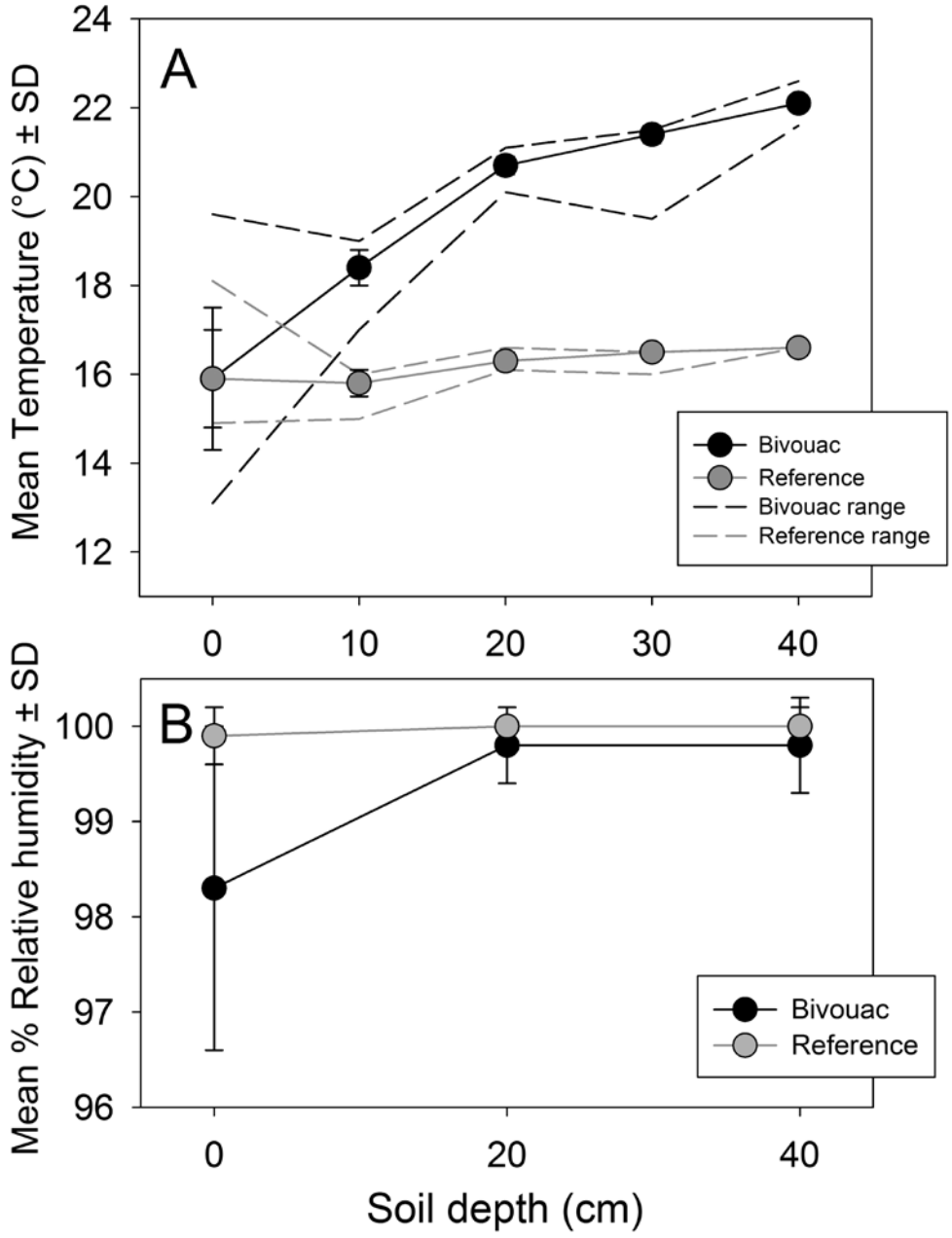

Figure 4-4. A. Bivouac and uninhabited (reference) soil temperatures at varying depths from the surface ± standard deviation; perforated lines represent maximum and minimum recorded temperatures across all five days; **B.** Bivouac and reference percent relative humidity at varying depths from the surface ± standard deviation

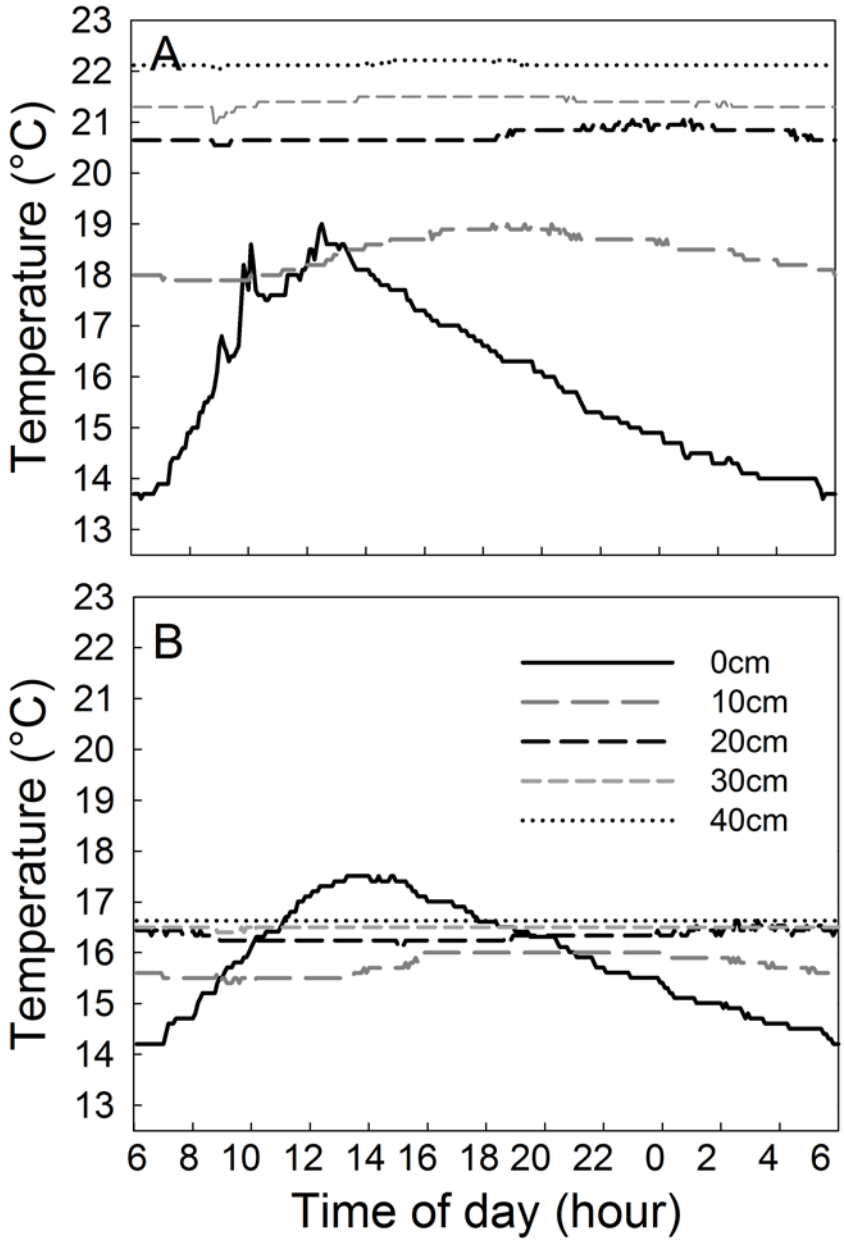

Figure 4-5. A. Bivouac , and **B.** reference temperatures across times of day, recorded every five-minutes and averaged across 5 days of recording; different lines denote probe depth in soil

CHAPTER 5: AMBIENT TEMPERATURE AND BROOD STAGE CONSTRAIN SOCIAL THERMOREGULATION OF ARMY ANT BIVOUACS (*Eciton burchellii parvispinum*)

Introduction:

Social thermoregulation & elevation

Thermoregulation is the maintenance of a narrow range of body temperatures (T_b) independent of variant ambient temperature (T_a), whereas T_b of thermoconformers covaries with T_a (Figure 1; Angilletta 2009). Similar to an individual endotherm composed of cells, thermoregulation can be an emergent property of insect societies composed of many thermoconforming individuals (Jones & Oldroyd 2006). Thermoregulation is a major adaptive feature of insect societies (Wilson 1971; Jones & Oldroyd 2006). Among social insects, social thermoregulation often serves the important function of incubating temperature-sensitive altricial brood (Franks & Sendova-Franks 1992; Jones *et al.* 2004; Penick & Tschinkel 2008; Becher, Scharpenberg & Moritz 2009). However, perfect thermoregulation and thermoconformation represent extremes on a spectrum. Individual organisms are sometimes intermediate, neither thermoconforming nor thermoregulating perfectly (Bauwens, Castilla & Mouton 1999). Environmental thermal variation drives patterns in thermal tolerances among ant workers (Diamond *et al.* 2012; Kingsolver, Diamond & Buckley 2013; Stuble *et al.* 2013; Baudier *et al.* 2015; Kaspari *et al.* 2015; Bishop *et al.* 2016), but little is known of how social thermoregulation varies across geographic thermal gradients. Tropical elevation gradients exhibit relatively little seasonal variation in temperature, causing higher rates of thermal speciation and more elevationally restricted species ranges than at high latitudes (Janzen 1967; Ghalambor *et al.* 2006). I used army ant bivouacs as models to ask how social homeostasis responds to temperature variation along a tropical elevational cline.

The bivouac: a model of social thermoregulation

The temporary nests, or bivouacs, of the army ant *Eciton burchellii* (Westwood) are thought to be a prime example of near-perfect social thermoregulation, with stable bivouac temperatures (T_{biv}) of 28.5±1°C regardless of T_a (Schneirla, Brown & Brown 1954; Franks 1989; Jones & Oldroyd 2006). However, all studies of *Eciton* bivouac thermoregulation to date have been conducted in relatively warm lowland tropical forests, where T_a is relatively similar to internal bivouac temperatures (Figure 1; Schneirla, Brown & Brown 1954; Jackson 1957; Teles da Silva 1977; Franks 1989). The army ant *E. burchellii* occupies a wide elevational range spanning at least 1700 m (O'Donnell *et al.* 2011), with an expected difference in annual temperature of approximately 16.7°C assuming 9.8°C adiabatic cooling/1000 m elevation gain (Lazaridis 2011). I asked whether and how the performance of bivouac thermoregulation responds to spatial variation in T_a at the two extremes of this range within Costa Rica: Guanacaste & Monteverde (Figure 1).

Army ant bivouacs are temporary nests composed entirely of interlocking worker bodies that cradle the brood and queen, warming them with collective metabolic heat via clustering (Schneirla, Brown & Brown 1954; Jackson 1957; Franks 1989; Jones & Oldroyd 2006). Bivouacs of the army ant *E. burchellii* are often constructed above ground, where they contact more variable ambient temperatures than underground bivouacs (Rettenmeyer 1963; Franks 1989; Soare *et al.* 2011; Baudier & O'Donnell 2016). The geographic range of *E. burchellii* consists of a mosaic of subspecies, with the northernmost *Eciton burchellii parvispinum* (Forel) occurring over the widest elevational range of 0 – 1700 m asl within Costa Rica (Watkins 1976; O'Donnell *et al.* 2011; Winston, Kronauer & Moreau 2017). These features make bivouacs of *E. burchellii parvispinum* particularly tractable models for testing hypotheses about social adaptation to climatic variability. I asked whether bivouacs of *E. burchellii parvispinum* at the lower and upper elevational extremes of their range in Costa Rica tightly thermoregulate to within the putative window of 28.5±1°C (Schneirla, Brown & Brown 1954; Franks 1989).

Active and passive thermoregulation

Active thermoregulation is the modification of internal temperature via physical or metabolic activity, while passive thermoregulation utilizes environmental temperature gradients to modify internal temperatures (Jones & Oldroyd 2006). Examples of active thermoregulatory mechanisms in social insects include wing fanning and clustering, while nest site choice and movement of brood within the nest are examples of passive social thermoregulation (Jones & Oldroyd 2006; Penick & Tschinkel 2008; Weiner *et al.* 2010; Soare *et al.* 2011). Wingless army ants are able to actively warm their brood via clustering (Jones & Oldroyd 2006), but whether active cooling exists in bivouacs has not been tested. By estimating active bivouac thermoregulation relative to T_a across the elevational range extremes of *E. burchellii parvispinum*, I explore whether and to what extent active warming or cooling is performed in response to elevationally driven differences in temperature.

Brood developmental stage and thermoregulation

Optimum growth temperature can differ with juvenile developmental stage (Jay 1963; Liu *et al.* 2005; Li *et al.* 2016). Social hymenopteran pupae tend to be more sensitive to thermal fluctuations and more tightly thermoregulated than larvae. In *Vespa orientalis* pupal caps cause more stable temperatures for pupae than for uncapped larvae via silk insulation (Ishay & Barenholz-Paniry 1995). The pupae of *Apis melifera* are more tightly thermoregulated than larvae (Kronenberg & Heller 1982), as pupal temperature deviations as little as 1°C can alter microglomerular development, resulting in cognitive and behavioral changes in adult workers (Groh, Tautz & Rössler 2004; Jones *et al.* 2005; Becher, Scharpenberg & Moritz 2009). Many social insects cope with differences in brood needs by separating brood spatially within the nest (Jay 1963; Franks & Sendova-Franks 1992) and by engaging in brood-specific thermoregulation behaviors such as fanning or direct incubation in the immediate vicinity of each brood type

(Heinrich 1975; Franks & Sendova-Franks 1992; Cook *et al.* 2016). Bivouacs of *E. burchellii parvispinum* are good models to test for brood-driven differences in thermoregulation because brood development is synchronized at the colony level (Supplementary figure S5-1). Colonies in the statary phase house pupae and eggs, and colonies in the nomadic phase house only larval brood (Rettenmeyer 1963; Gotwald Jr 1995). In this way, a bivouac typically only meets the needs of either larvae or pupae and eggs at a single point in time. I predicted that pupal bivouacs would be more tightly thermoregulated than larval bivouacs, and I asked whether this relationship varied with T_a across elevations. I compared overall bivouac temperatures between colony phases and bivouac temperatures at the site of the brood to determine whether colony movement drives bivouac-wide differences in temperature, or if there are brood-local differences in thermoregulation within the bivouac.

Bivouac relative humidity

Adult *E. burchellii* workers show signs of distress when exposed to relative humidities of 76% or lower (Schneirla, Brown & Brown 1954). However, immature social insects often have higher desiccation risk than adult nestmates (Lindauer 1955; Human, Nicolson & Dietemann 2006; Ellis *et al.* 2008). I asked whether and to what degree relative humidity (RH) is maintained within bivouacs and whether ambient RH (RH_a) affects bivouac RH (RH_{biv}). By measuring RH_{biv} across seasonal dry forest and wet forest, I present the first descriptions of bivouac humidity.

Within-bivouac temperature range

Perfect thermoregulation is the maintenance of both a particular mean T_b as well as a particular variance in T_b in a variable environment (Angilletta 2009). For bivouacs, this means both directional modification of mean T_{biv} from mean T_a, and also reduced variation in T_b relative to T_a variation. I asked whether thermal variation within bivouacs is less than T_a variation and whether thermal buffering differs across elevational extremes.

Bivouac site selection

Nest site selection and orientation are passive mechanisms of thermoregulation used by many social insects (Heinrich 1975; Heinrich 1993; Penick & Tschinkel 2008; McGlynn *et al.* 2010). Because army ants do not construct permanent nests, colonies must frequently assess and select new bivouac sites from an array of novel prospects. Below-ground bivouacking army ant species may utilize passive thermal buffering effects of soil to reduce variation in bivouac temperature (Baudier & O'Donnell 2016). I hypothesized bivouac site selection is an elevationally variable form of passive thermoregulation. I predicted *E. burchellii parvispinum* would select relatively cooler bivouac sites at its low elevation (high T_a) range limits, while selecting relatively warmer bivouac sites at its high elevation (low T_a) range limits (Soare *et al.* 2011). I also predicted bivouac sites with higher RH would be selected in the lowlands at Santa Rosa where RH_a is relatively low.

Geographic surveys suggested *E. burchellii* bivouacs are more likely to be placed in sheltered and subterranean locations and at high elevations during the statary phase (Schneirla, Brown & Brown 1954; Rettenmeyer 1963; Soare *et al.* 2011). However, the low and high elevation data sampled different *E. burchellii* subspecies. I asked whether bivouac site choice (exposure or tendency to be below ground) differs across the maximum elevation range of *E. burchellii parvispinum*. I predicted this subspecies is less likely to bivouac aboveground or exposed than other *E. burchellii* subspecies, as it occupies more arid and variable ambient conditions across its geographic range (Watkins 1976).

Methods

Study sites and colony tracking

Two main field sites at the elevational extremes of the *E. burchellii parvispinum* range in Costa Rica were used for this study: high-elevation Monteverde (N10°18', W 84°49', sampled 1160-1602 m asl) and low-elevation Guanacaste (Santa Rosa National Park and Maritza Biological station, N10°53', W85°46', sampled 268-312 m asl; N10°57', W85°29', sampled 402-607 m asl). Within each site I located bivouacs of *E. burchellii parvispinum* by trail-walking methods (Kumar & O'Donnell 2009; Soare *et al.* 2011). Army ant raid columns were followed in the direction of carried food items to reach bivouacs. Bivouacs were checked nightly for signs of emigration. Emigrations were followed to each new bivouac site to identify successive bivouacs of the same colony whenever possible. Geographic coordinates and elevation were measured using handheld GPS units (Garmin CPSMAP® 62S, Garmin International Inc., Olathe, KS, USA) and were recorded for each bivouac to the nearest 10 m. Bivouacs encountered more than 300 linear meters apart within 24 hours of each other were assumed to be different colonies, as this exceeds maximum recorded length of a single overnight emigration (Teles da Silva 1982). Data were collected in the wet season of 2013 (24-27 July2013, Monteverde, N=1 bivouac), in the wet season of 2015 (10 June -11July 2015, Monteverde, N=36 bivouacs; 12July -13 August 2015: Santa Rosa: N=32 bivouacs), and in the transition from dry season to wet season of 2016 (5 April – 1 May 2016 Monteverde N=19 bivouacs; 2 May – 16 May & 19 May - 2 June 2016 Santa Rosa, N= 10 bivouacs; 16 May -19 May 2016 Maritza, N=6 bivouacs).

Statistical analyses

All statistical analyses were performed in R (version 3.1.2, R Development Core Team 2011). Colony identity was included as a random predictor variable in all statistical models testing

effects on bivouac conditions, but I present colony effects only when they were significant. % Relative humidity was arcsine transformed in all analyses.

Ambient conditions and elevation

Effects of elevation on T_a were tested by regressing elevation with mean T_a (measured at forest floor 1m from bivouacs). I used 95% confidence intervals to compare the slope of T_a versus elevation to the predicted adiabatic cooling of 9.8°C every 1000 m. Student's t-tests were used to compare T_a and RH_a between Guanacaste and Monteverde in separate analyses.

Bivouac temperature and humidity

Ibutton Thermochron and Hygrochron data loggers were used to record temperature and RH within and outside bivouacs (Maxim Integrated™, San Jose, CA, USA). Ambient control probes measured conditions on the forest floor beneath leaf litter approximately 1 m away from each bivouac site. Bivouac probes consisted of four loggers placed 5 cm apart along a thermally inert plastic dowel starting from the apical tip. When RH was measured for the bivouac, a hygrochron was placed at the apical position. Prior to probe insertion, a small observation hole was made in the bivouac and brood depth measured to the nearest centimeter using a ruled dowel. Logger probes were positioned within the bivouac so that brood surrounded the apical tip of the probe. The bivouac was allowed to re-form around the probe then the bivouac was given an extra 30 minutes for instrument equilibration before logs were counted. Loggers ran 24 hours a day collecting temperature logs, or temperature and RH logs, every 5 minutes. Changes in bivouac volume and relative probe position were monitored using time-lapse PlotWatcher Pro game cameras (Day 6 Outdoors, Columbus, GA, USA). Thermal conditions of each bivouac were recorded for up to five days, shorter for bivouacs that emigrated before the end of 5 days. Most nomadic bivouacs were measured 1 to 2 days due to frequent emigration. Mean T_{biv}, RH_{biv}, T_a, RH_a, and within-colony standard deviations were calculated across days, times of day, and across

probes (in the case of T_{biv}) at each bivouac site. Logs recorded during bivouac emigrations were excluded from all analyses.

Linear subset models were fitted that included mean T_{biv} as a response variable, and mean T_a, colony phase, and colony ID as predictor variables; factors were eliminated until the minimum required model was reached (Quinn & Keough 2002). Akaike information criterion was used to direct order of elimination. A separate analysis was used to test significance of predictors for RH_{biv}. Linear subset models were fitted that included mean arcsine transformed mean RH_{biv} as a response variable, and arcsine transformed mean RH_a, colony phase, and colony ID as predictor variables; factors were eliminated until the minimum required model was reached as described above.

Temperature range was calculated as the maximum temperature minus minimum temperature measured at each bivouac, for both T_a and T_{biv}. A linear subset model with temperature range as response variable was fitted to include colony phase, elevational site (Monteverde versus Santa Rosa), and whether the range was of T_{biv} or T_a. Factors were eliminated until the minimum required model was reached. A post-hoc Tukey test was used to delineate differences in temperature range between bivouac and ambient, across Monteverde and Santa Rosa.

Brood temperatures were a subset of the bivouac thermal measurements. After equilibration time following probe insertion, temperature logs for one hour (N=12 logs) across the depths at which brood had been observed within the bivouac were considered to be representative of the brood conditions. Brood type was recorded and generally corresponded to phase predictably, with some early nomadic and late statary bivouacs housing both pupae and larvae. An ANCOVA was used to test for differences between larvae and pupae in the relationship between mean T_a and mean T_{biv}. A separate ANCOVA was used to test for differences between larvae and pupae in the relationship between mean RH_a and mean RH_{biv}.

Passive site temperature and humidity

Bivouac site conditions without ants were monitored by leaving all probes in place after each colony had emigrated. Control ambient temperatures were simultaneously collected from a reference probe on the forest floor 1 m from the abandoned bivouac site. Data were recorded minimum 8 hours, maximum 24 hours after each bivouac had emigrated. A linear regression between mean bivouac site temperature and mean ambient temperature was performed to test for effects of T_a on empty bivouac site temperature (T_{site}). RH_a and empty bivouac site relative humidity (RH_{site}) were similarly regressed in a separate analysis.

Active warming

Active warming caused by collective metabolic heat of ants and brood (T_w) was estimated as:

$$T_w = \bar{T}_{biv} - \bar{T}_{site}$$

where $\bar{T}_{biv}$ was mean bivouac temperature while ants were present, and $\bar{T}_{site}$ was mean bivouac site temperature after ants had emigrated. T_w was regressed against T_a to test whether active bivouac warming differed across elevations. This assumes changes in T_a before and after bivouacs emigrated did not affect within-cavity conditions. In order to test whether these effects were major factors in observed patterns, I ran the same analyses on a subset of time-matched data points for which changes in average T_a were less than or equal to 3°C (N=13). Both the subset and the original data set were compared using linear models with colony ID and T_a as predictors of T_w.

Bivouac site-type survey

Bivouacs were scored for relative exposure and above-groundedness in Monteverde and Guanacaste for a total of two months at each site. Fifty-five bivouacs were observed in Monteverde in June of 2015 and April of 2016, and 48 bivouacs were observed in Guanacaste in July 2015 and May 2016.

An exposed bivouac was considered to be one which has ≥50% visible surface exposed to the environment (Schneirla, Brown & Brown 1954; Soare *et al.* 2011). I distinguished two separate categories of non-exposed bivouacs: bivouacs were considered to be "enclosed" if no portion of bivouac surface was observable from the exterior, while "sheltered" bivouacs were partly observable from the surface, but less than 50% exposed to the elements. Whether bivouac sites were above or below ground was also recorded.

A screening 4x3 Chi squared analysis was performed to test for effects of colony phase and elevation (4 combinations as predictors) on the frequency of all three exposure categories (exposed, sheltered, and enclosed). A screening 4x2 Chi squared analysis was performed to test for effects of colony phase and elevation (4 combinations as predictors) on the frequency of bivouac soil microhabitat use categories (subterranean versus aboveground). Subsequent post-hoc Chi square analyses were performed to test within each category if effects were found.

Results

Ambient conditions across elevations and sites

Ambient temperature decreased as elevation increased across sampled sites (Figure 1; $F_{1,35}$=420.4, R^2=0.92, p<0.001; y=29.07-0.0073x). Predicted adiabatic cooling 9.8°C/1000m fell outside of the 95% confidence interval of (0.0073±0.0007). T_a was higher in Guanacaste than in Monteverde (t=17.91, df=35, p<0.001). RH_a was lower in Guanacaste than in Monteverde (t=6.66, df=19, p<0.001).

Bivouac and brood conditions

Mean bivouac temperature decreased with mean decreasing T_a (Figure 2; $F_{1,34}$=25.04, R^2=0.41, p<0.001, y=17.45+0.37x). Colony phase was not a significant predictor of mean bivouac temperature ($F_{1,33}$=2.89, p=0.099). As T_a increased, so did mean temperature of larvae ($F_{1,19}$

=21.21, R^2=0.50, p<0.001, y=14.09+0.50x) and mean temperature of pupae (Figure3; $F_{1,15}$=7.53, R^2=0.29, p=0.015, y=21.75+0.21x). However, pupal temperature varied significantly less with T_a than did larval temperature (Figure 3; $F_{1,34}$=4.26, p=0.047).

The relationship between T_{biv} range and T_a range for individual bivouacs differed with elevation (Figure 4; $F_{1,68}$=12.84, p<0.001), but temperature range did not differ between nomadic and statary bivouacs ($F_{1,67}$= 2.86, p=0.095). T_{biv} varied less than T_a in Santa Rosa (t=2.95, p=0.022), but Monteverde T_{biv} variability did not differ from T_a variation (t=2.06, p=0.176), even though T_a range in Monteverde was smaller than in Santa Rosa (t=2.95, p= 0.022).

Mean RH_{biv} was above 86% in all observed bivouacs and was independent of ambient relative humidity (Figure 2; X^2=2.06, df=1, p=0.15) in models accounting for significant random effects of colony ID ($F_{2,14}$=41.98, p=0.024). Colony phase was also not a significant predictor of RH_{biv} (X^2=0.01, df=1, p=0.90). Ambient relative humidity was not a significant predictor of relative humidity at the site of brood within the bivouac ($F_{1,13}$=0.544, p=0.47). Larvae and pupae did not differ in relative humidity ($F_{1,14}$=1.06, p=0.32). Mean relative humidity at the site of brood ± standard deviation was 93.34 ± 5.53 % (N=20 bivouacs) across all bivouacs and elevations.

Empty bivouac site conditions

Temperature within evacuated bivouac sites was strongly predicted by T_a (Figure 2; $F_{1,14}$=142.3, R^2=0.90, p<0.001; y=5.15+0.74x). However the slope of the regression line was significantly less than 1 (slope ± 95% confidence interval was 0.743 ± 0.122), suggesting warm site selection when T_a was low and cool site selection when T_a was high. Bivouacs were more moist than ambient, but mean RH_{site} decreased with RH_a, (Figure 2; $F_{1,8}$=15.13, R^2=0.61, p=0.005).

Active warming and elevation

All mean bivouac temperatures were greater than or equal to (within instrument error ± 0.5°C) their corresponding mean empty site temperatures (Figure 5; N=22). Active warming decreased

as T_a increased (Figure 5; $F_{1,14}$=22.90 , R^2=0.59, p<0.001; y=15.26-0.47x). To test whether this pattern was affected by changes in T_a between bivouac data collection days and subsequent site measurement days, I re-ran the analysis on a subset (N=13) of bivouac data points for which all temperatures for the bivouacs and the empty sites were time-of-day matched. Mean T_a change among days was less 3°C; the analysis yielded a similar inverse relationship between ambient temperature and estimated active thermoregulation ($F_{1,11}$=10.50, R^2=0.44, p=008; y=13.34–0.40x).

Bivouac site survey

Of 103 surveyed bivouacs in 2015 and 2016, 24.3% were exposed, 28.2% were sheltered, and 47.6% were enclosed. There was no effect of either colony phase or elevation on proportion of bivouacs that were exposed, sheltered, or enclosed (Figure 6; X^2 =2.85, df=6, p=0.83). Bivouacs were more frequently subterranean in Santa Rosa compared to Monteverde (Figure 6;X^2=10.16, df=1, p=0.001). Frequency of subterranean bivouacs did not differ between nomadic and statary bivouacs (X^2=0.02, df=1, p=0.876).

Discussion

Elevation and bivouac temperature

Our data show that *E. burchellii parvispinum* bivouacs thermoregulate imperfectly over their elevationally driven T_a range. Bivouacs at high elevation sites were significantly cooler overall. Reduced bivouac temperatures at high-elevation brood cores show that this pattern is directly felt by developing larvae and pupae. Decreased immature temperatures slow time to maturity among many insects (Williams 1946; Porter 1988; Abril, Oliveras & Gómez 2010). Cooler mean bivouac temperatures may cause increased brood developmental time and alter the *E. burchellii parvispinum* colony developmental cycle, though I did not test for these effects. Cooler brood temperatures may limit higher elevation distribution of these ants. Both within and between

bivouac temperature variation was also higher in Monteverde despite T_a variation being less in Monteverde than in Guanacaste. Although brood-stage differences accounted for some of the bivouac temperature variation, both larval and pupal temperatures varied more in Monteverde than in Santa Rosa. What the relative impacts are of high thermal variation versus non-ideal mean temperatures in terms of brood development remains an interesting question in considering tradeoffs between thermoregulation strategies.

In relatively dry and hot lowland Santa Rosa, mean T_a frequently exceeded the putative thermoregulation window of 28.5±1°C for *E. burchellii* bivouacs (Franks 1989), with mean forest floor temperatures as high as 30.5°C in the immediate vicinity of bivouacs. Bivouacs do not maintain a narrow homeostatic thermal range across elevations, but my data show that bivouacs, particularly measured at brood cores, are not typically hotter than 28–29°C at any elevation. I suggest these temperatures may be at or near the upper limit for normal brood development of *E. burchellii parvispinum*. This is cooler than upper thermal limits of brood in other actively thermoregulating Hymenoptera: 36°C for pupae and 37°C for larvae in *A. melifera* (Himmer 1932; Becher, Scharpenberg & Moritz 2009). Brood upper thermal limits below that of temperatures common in the environment suggest high susceptibility of *E. burchellii parvispinum* brood to increasing temperatures associated with anthropogenic climate change. Adult worker thermal tolerance is not likely challenged within bivouacs however, as even small caste adult thermal tolerance of *E. burchellii parvispinum* tends to be over 35°C (Baudier *et al.* 2015).

Brood developmental stage differences

Though both brood stages experienced similar temperatures in lowland Santa Rosa, pupae tended to be kept at higher and less variable temperatures than larvae in high-elevation Monteverde. Overall bivouac temperature did not differ across colony phases. This suggests that thermoregulation differences are fairly localized to areas of the bivouac where brood is housed.

Though other ants separate brood stages spatially in order to cater to differences in thermal needs (Franks & Sendova-Franks 1992), army ants separate brood thermoregulation temporally via developmental synchrony. Though the evolution of developmental synchrony in army ants is most commonly discussed in terms of brood and adult nutritional needs (Kaspari *et al.* 2011), my data suggest the need for different thermoregulation strategies between larvae and pupae may have also played a role in the evolution of this behavior in thermally heterogeneous habitats.

Bivouac site selection

While winged social insects use fanning to cool overheated broods (Jones *et al.* 2004; Cook *et al.* 2016), wingless ants generally lack active cooling mechanisms (Hölldobler & Wilson 1990). Army ants likely rely heavily on passive thermoregulation in the form of selecting cool and moist underground bivouac cavities to lower bivouac temperatures in hot environments. In the lowlands selected bivouac sites were significantly cooler than T_a, while bivouac sites significantly warmer than T_a were selected at high elevations. Though site selection did contribute to bivouac warming at high elevations, the warming effect was small with ant presence playing a larger role in heating than did site alone.

Bivouac exposure for *E. burchellii parvispinum* did not vary with elevation or colony phase, with an average 24.3% of bivouacs exposed across elevations. This is in stark contrast to previous observations across multiple *E. burchellii* subspecies in Central and South America (Soare *et al.* 2011). My survey did not support the hypothesis that subterranean bivouacs at high elevations are a plastic behavioral mechanism of thermoregulation. Rather, sheltered bivouac sites may be a fixed trait in *E. burchellii parvispinum. Eciton burchellii parvispinum* is a monophyletic lineage that is the sister taxon to all other *E. burchellii* subspecies (Winston, Kronauer & Moreau 2017). The known elevational range of *E. burchellii parvispinum* is greater than other subspecies, also spanning into more arid and seasonal landscapes (Watkins 1976;

O'Donnell *et al.* 2011). Exposed bivouacs may have higher rates of convective cooling (Soare *et al.* 2011), but exposure likely also subjects bivouacs to higher rates of desiccation in arid regions of northern Central America. Bivouacs that are fully enclosed are also likely less susceptible to diel fluctuations in temperature due to buffering effects of enclosing cavities.

Bivouacs of *E. burchellii parvispinum* in Monteverde were less likely to be underground than in lowland Guanacaste. This pattern was also contrary to cross sub-species elevational patterns (Soare *et al.* 2011). Lowland secondary forests of Guanacaste saw larger diel fluctuations in ambient relative humidity and temperature than did Monteverde. Soil buffering plays an important role in shielding underground bivouacs of other army ants from extreme temperatures associated with diel thermal fluctuations (Baudier & O'Donnell 2016) and may be used facultative by *E. burchellii parvispinum* for this purpose. The tendency to bivouac below ground in Santa Rosa may better shield from desiccation during daytime drying as well.

Hygroregulation

In Monteverde RH_a was typically over 80% throughout the day even in later portions of the dry season. Santa Rosa RH_a was much more variable, commonly dropping below 50% at mid-day on days without rain. Though RH_{site} decreased slightly with RH_a, RH_{biv} was independent of mean RH_a and remained above 85% even for bivouacs where mean RH_a was as low as 60%. Together this suggests that bivouacs may passively hygroregulate via site selection, but that ants actively moisten their warm bivouacs additive to any passive effects of site.

Active warming and elevation

Taking effects of effects of site selection into account, the magnitude of active warming ($T_w = T_{biv} - T_{site}$) was highly responsive to T_a and decreased at low elevation sites. Mean bivouac temperature was actively elevated from that of bivouac site as much as 10°C and as little as 0°C. Negligible bivouac warming observed in some bivouacs goes against Franks' suggestion that

army ants have a baseline obligate warming load (1989). What mechanisms are involved in this responsive and variable active bivouac warming merit further study.

Notably, I observed no signs of net active cooling ($T_w < 0$) even when ambient forest floor temperature was relatively high. This suggests that passive mechanisms such as site selection are the major driver for bivouac cooling in environments where mean T_a is greater than 29°C.

Climate change implications

Dry forest populations of *E. burchellii parvispinum* in Santa Rosa may be at particular risk of extirpation with increased mean temperatures, as evidenced by their inability to actively cool and reliance upon availability of cool bivouac sites. As temperatures in Guanacaste increase, availability of cool potential bivouac sites will likely be reduced. Conversely, at high elevations increasing temperatures may trigger upwards elevational shifts into habitats previously uninhabited by forest-floor carpet raiding species, facilitated by increase in available warm bivouac sites and higher overall T_a.

Along with altering a single species' interaction with its abiotic environment, directional climate change also changes interspecific interactions within a forest community (Diamond *et al.* 2016). This is especially pertinent in communities where army ants occur, as *Eciton burchellii* is host to the most complex set of animal associations known to science (Rettenmeyer *et al.* 2011). Inquilines living within bivouacs can be more thermally sensitive to extreme temperature than their foraging ant hosts (Baudier & O'Donnell 2016). These complex communities are therefore at risk of direct negative effects due to extirpation of *E. burchellii parvispinum* from its historic range and may also be susceptible to costs incurred prior to extirpation, depending on specific breadths of thermal performance.

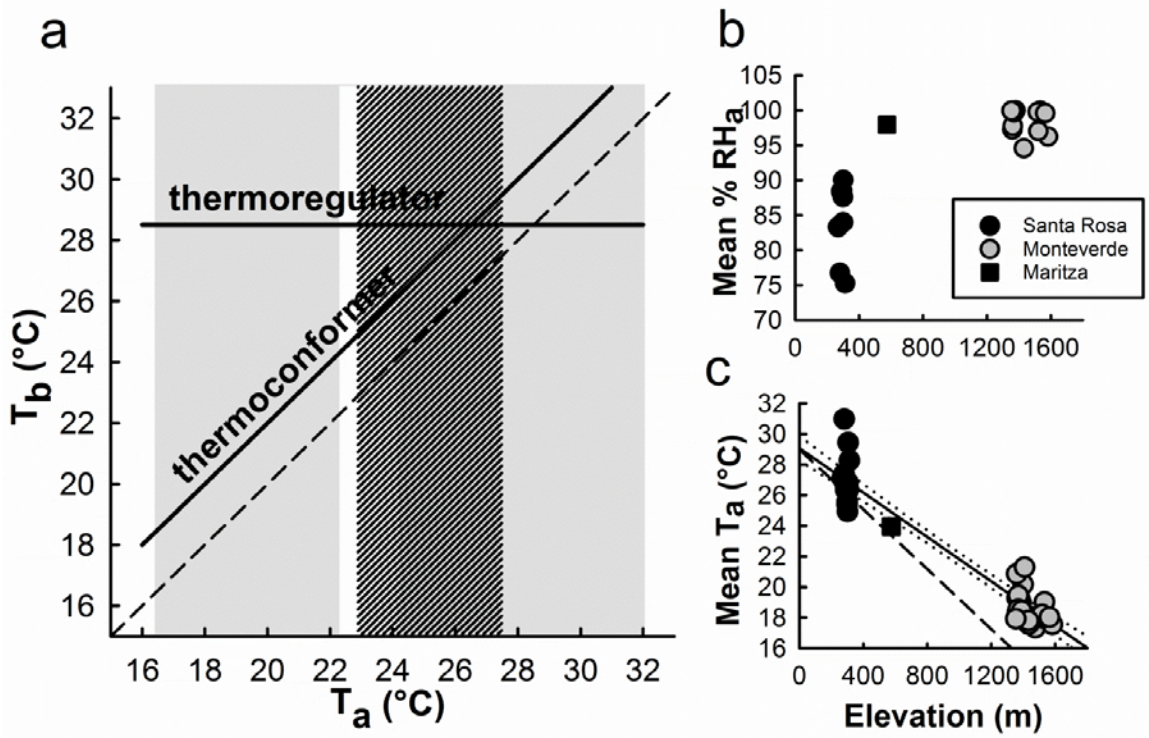

Figure 5-1. A. The relationship between ambient temperature (T_a) and internal temperature (T_b) for a putative perfect thermoregulator (slope = 0) and thermoconformer (slope = 1); hashed area is the lowland span of mean T_a where above-ground bivouac thermoregulation has been previously studied (Schneirla, Brown & Brown 1954; Jackson 1957; Franks 1989); grey boxes represent T_a sampled in this study at the elevational extremes where *Eciton burchellii parvispinum* occurs **B.** Ambient relative humidity across elevations and sites **C.** T_a across elevations and sites; solid line is regression, dotted line is 95% confidence interval of the regression; dashed line is predicted adiabatic cooling of 9.8°C per 1000 m

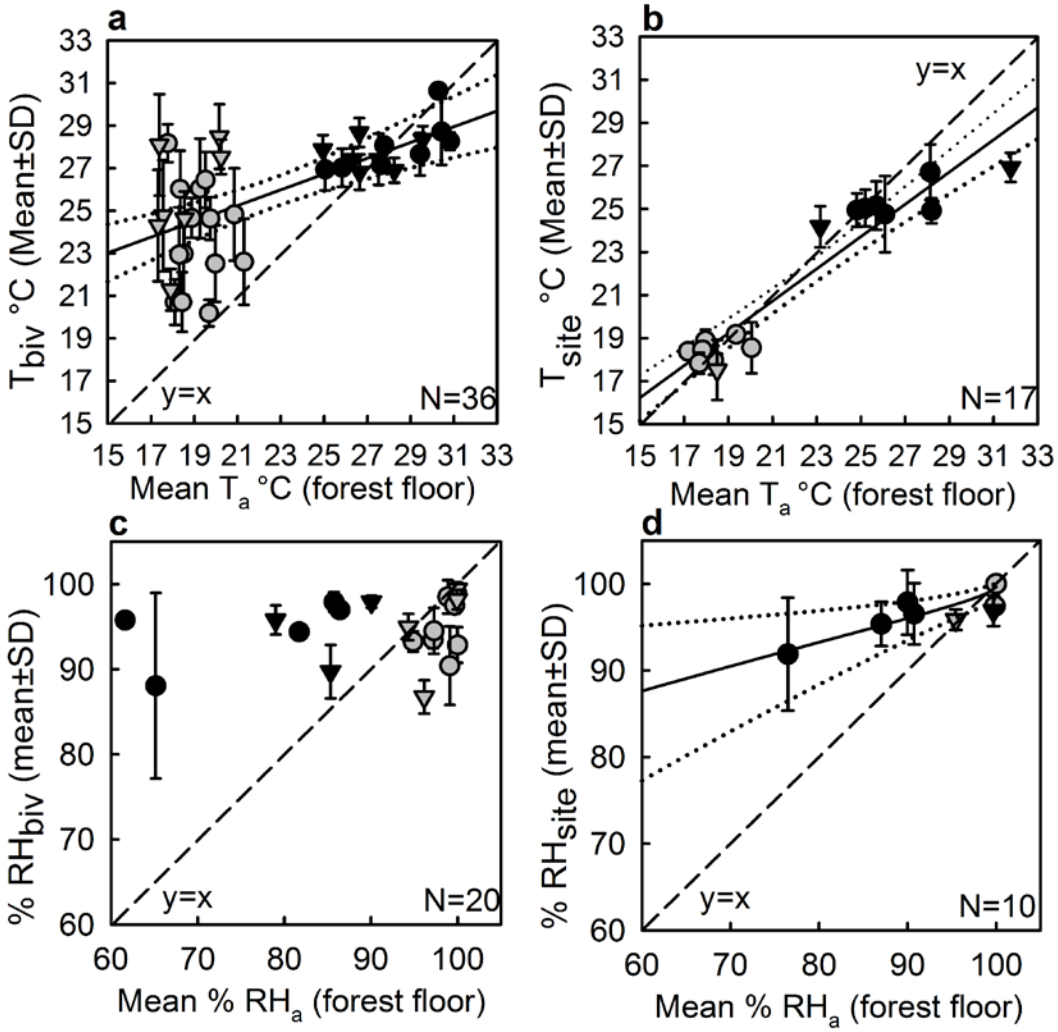

Figure 5-2. Relationship between mean ambient conditions and mean bivouac or site conditions at two elevations across all times of day; gray points = high elevation Monteverde, black points = low elevation Santa Rosa; triangles represent statary bivouacs, circles are nomadic bivouacs; N = total number of bivouacs sampled; 95% confidence intervals of bivouac-mean regressions depicted as dotted lines; standard deviations of within-bivouac variation depicted as error bars; **A & B** temperature, **C & D** relative humidity, **A & C** recordings from within present bivouacs, **B & D** recordings after bivouacs have emigrated out of each bivouac site; **D** trend and 95% confidence interval depicted from arcsine transformed relative humidity data

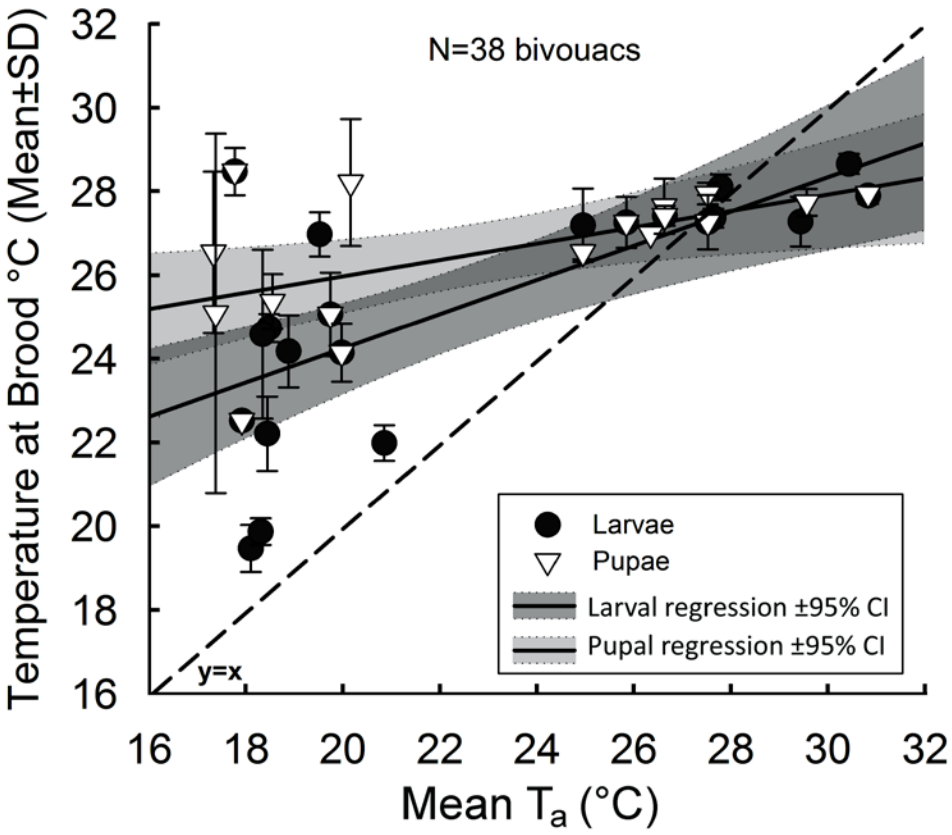

Figure 5-3. Effect of brood type on relationship between ambient and bivouac temperature at location of brood; error bars represent standard deviation of within-bivouac temperature variation; regressions and 95% confidence interval based on bivouac mean temperature at site of brood

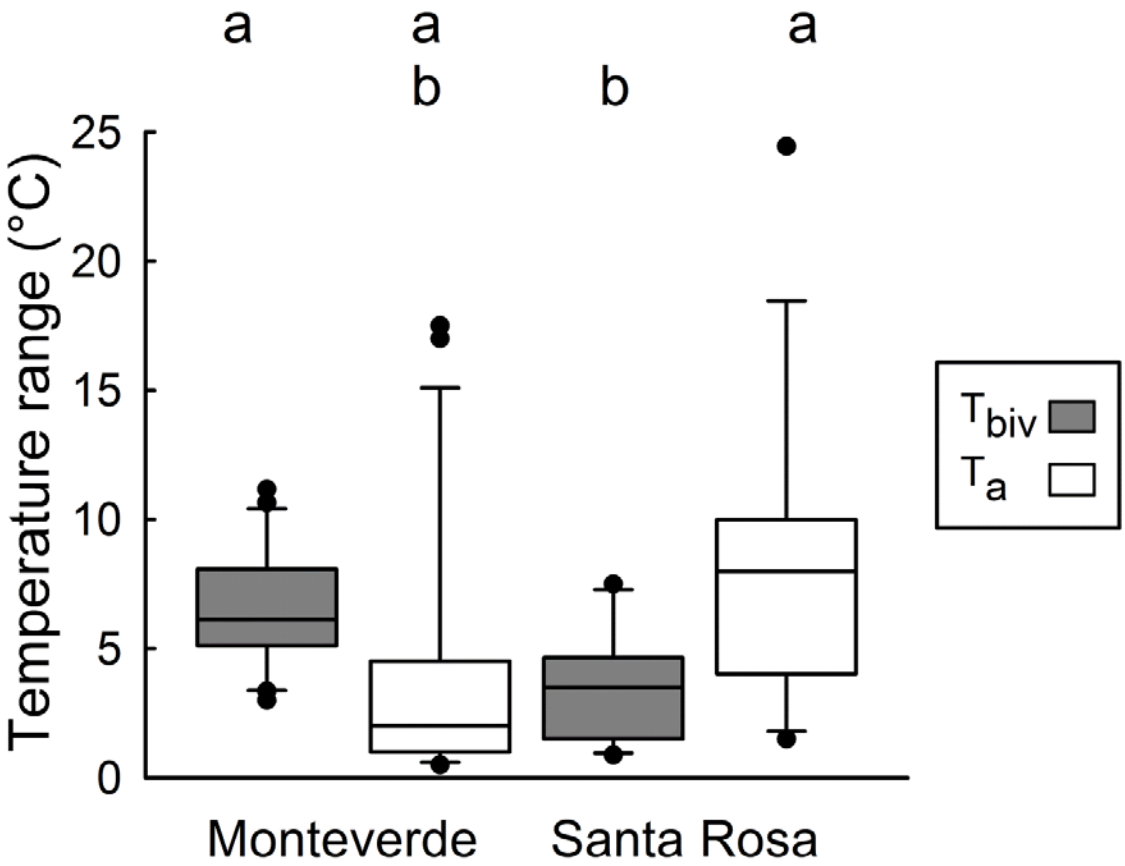

Figure 5-4. Within-bivouac temperature range (grey) and ambient temperature range (white) at high elevation Monteverde and low-elevation Santa Rosa; range was calculated as maximum temperature – minimum temperature

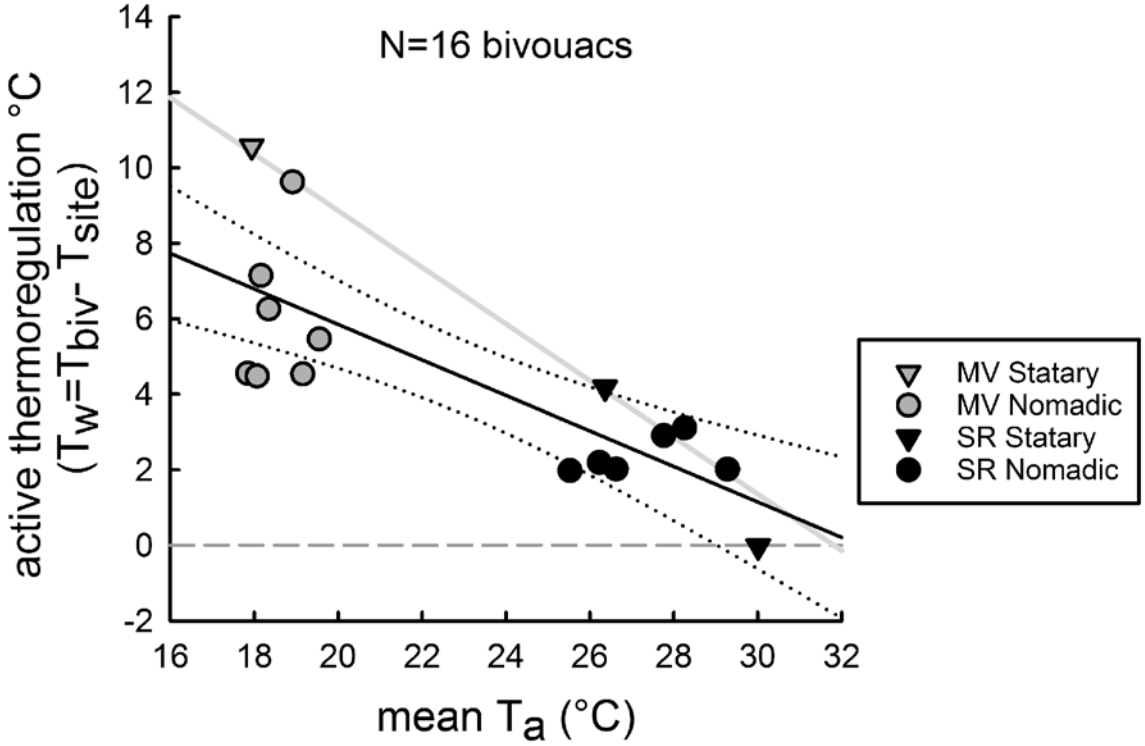

Figure 5-5. Relationship of mean ambient temperature with estimated active bivouac warming; dotted lines are 95% confidence intervals; dashed grey line is y=0 above which net active warming is observed and below which net active cooling is observed; the grey solid line is the thermoregulation required to maintain a homeostatic temperature of 28.5°C

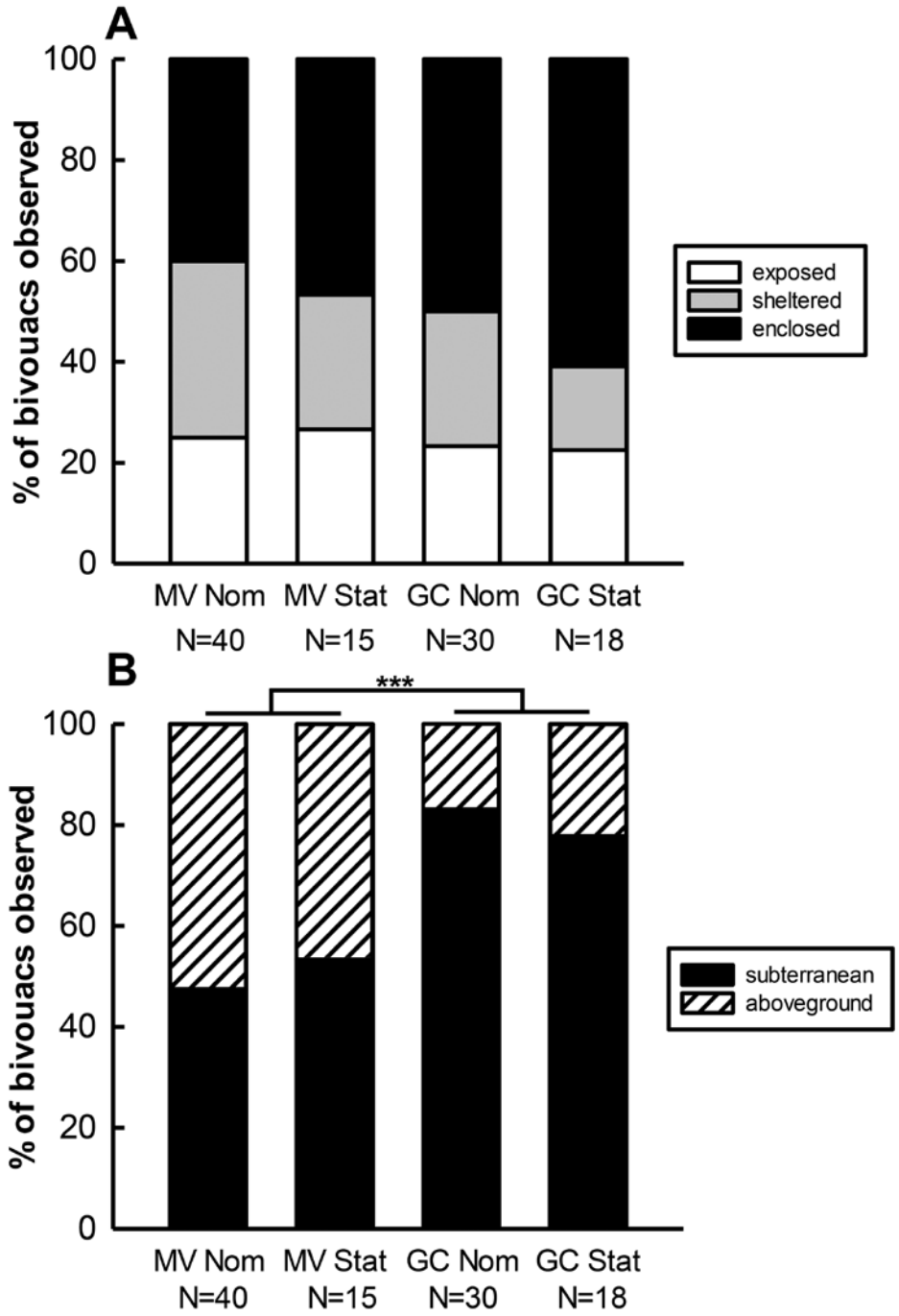

Figure 5-6. **A.** Bivouac exposure and **B.** % bivouacs above versus below ground across site (MV=Monteverde, GC=Guanacaste) and colony phase (Nomadic versus Statary) for 103 bivouacs surveyed in 2015 and 2016

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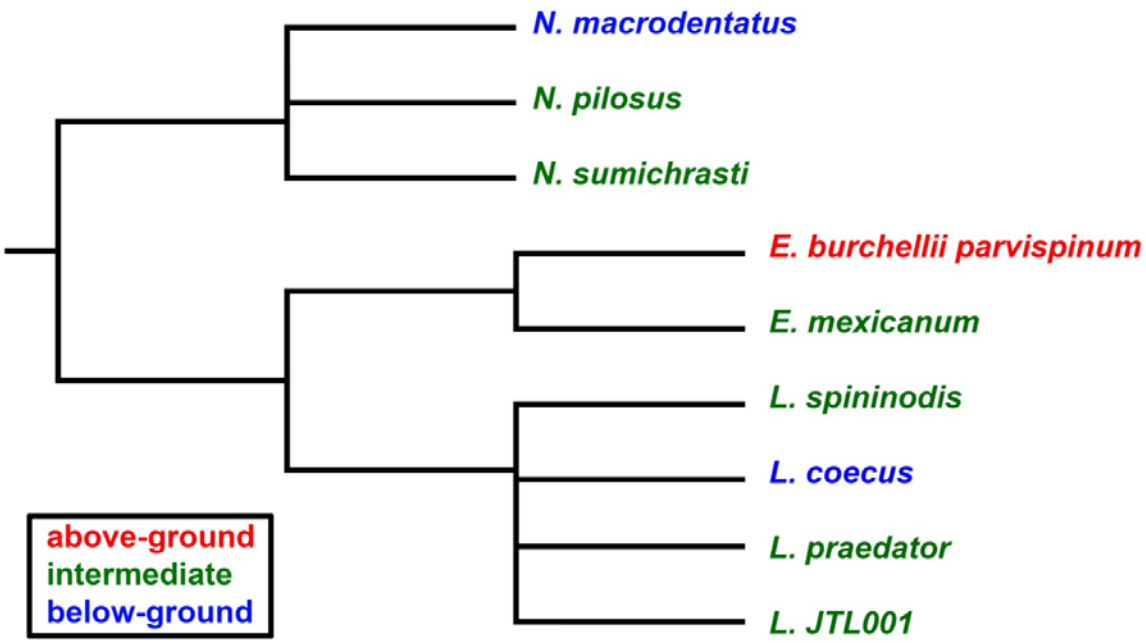
APENDECES

APENDIX A: Supplementary material for chapter 2

Supplementary Table S2-1. Summary of maximum infrared temperature readings taken at raids of several above-ground foraging army ants

Species	N Raids	Mean max raid T (°C)	SD max raid T (°C)	Max raid T (°C)	% $CT_{max} \leq$ max raid T
E. burchellii parvispinum	23	19.74	3.30	30	0.56%
E. mexicanum	2	16.85	2.05	18.3	0%
L. praedator	2	22.45	8.98	28.8	0%
L. JTL001	3	19.23	0.85	20.1	0%
N. sumichrasti	10	19.09	6.03	36.1	11.82%
Grand Total	**40**				



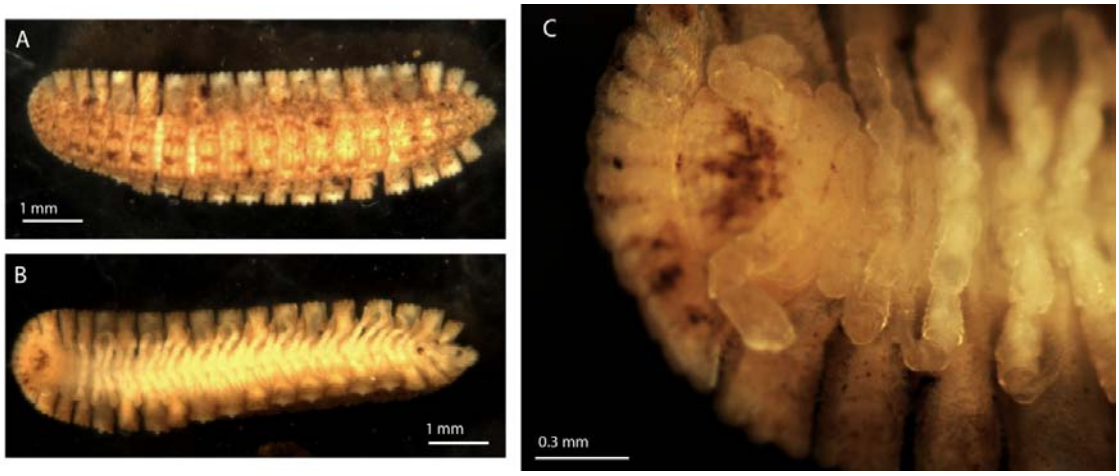
Supplementary Figure S2-1. Phylogeny used for comparative analysis; generic-level separation based on phylogenies (Brady 2003, Brady 2014)

APENDIX B: Supplementary material for chapter 4

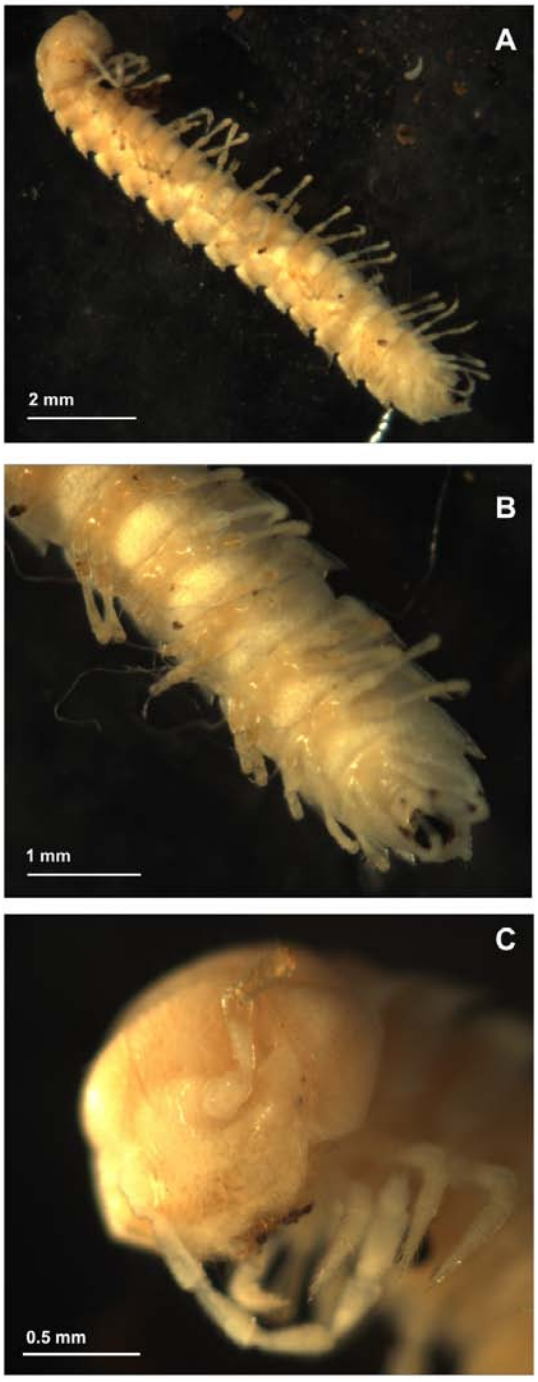
Supplementary Figure S4-1. Surface nest structure of three *Labidus praedator* bivouacs: **(A.)** bivouac A at 1500 m asl on 11 July 2014, **(B.)** bivouac B at 1565 m asl on 19 March 2015, **(C.)** bivouac C at 950masl on 22 April 2016.

Supplementary Figure S4-2. Surface of bivouac B covered in debris including discarded pupal cases and isopod tergites

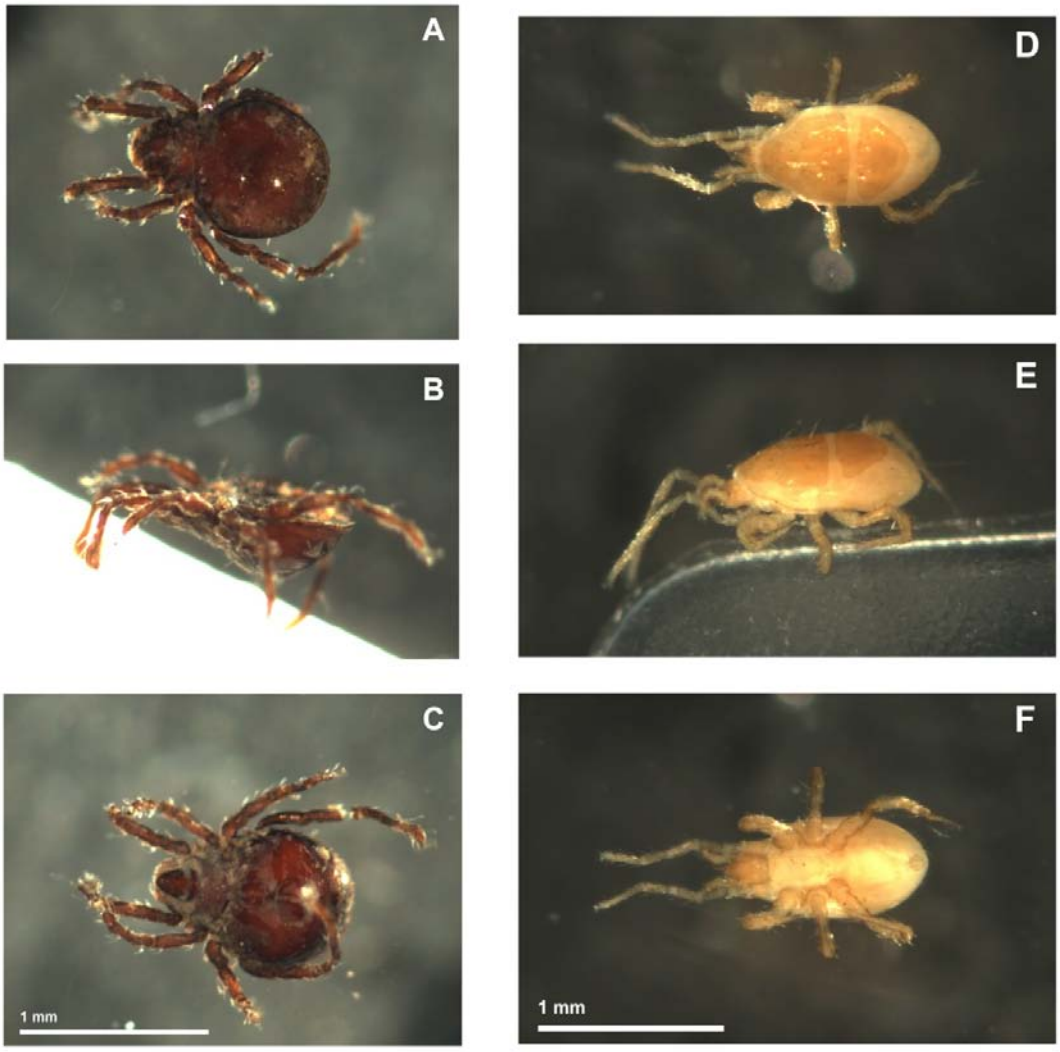
Supplementary Figure S4-3. A. Active bivouac A **B.** Active bivouac B, **C.** Collapsed former site of bivouac A in June of 2015, **D.** Collapsed former site of Bivouac B in June of 2015



Supplementary Figure S4-4. Voucher specimen of inquiline millipede species (*Calymmodesmus sp.*) subjected to thermal tolerance assays alongside host ants viewed dorsally **A.** ventrally **B.** and with close-up ventral view of head **C**



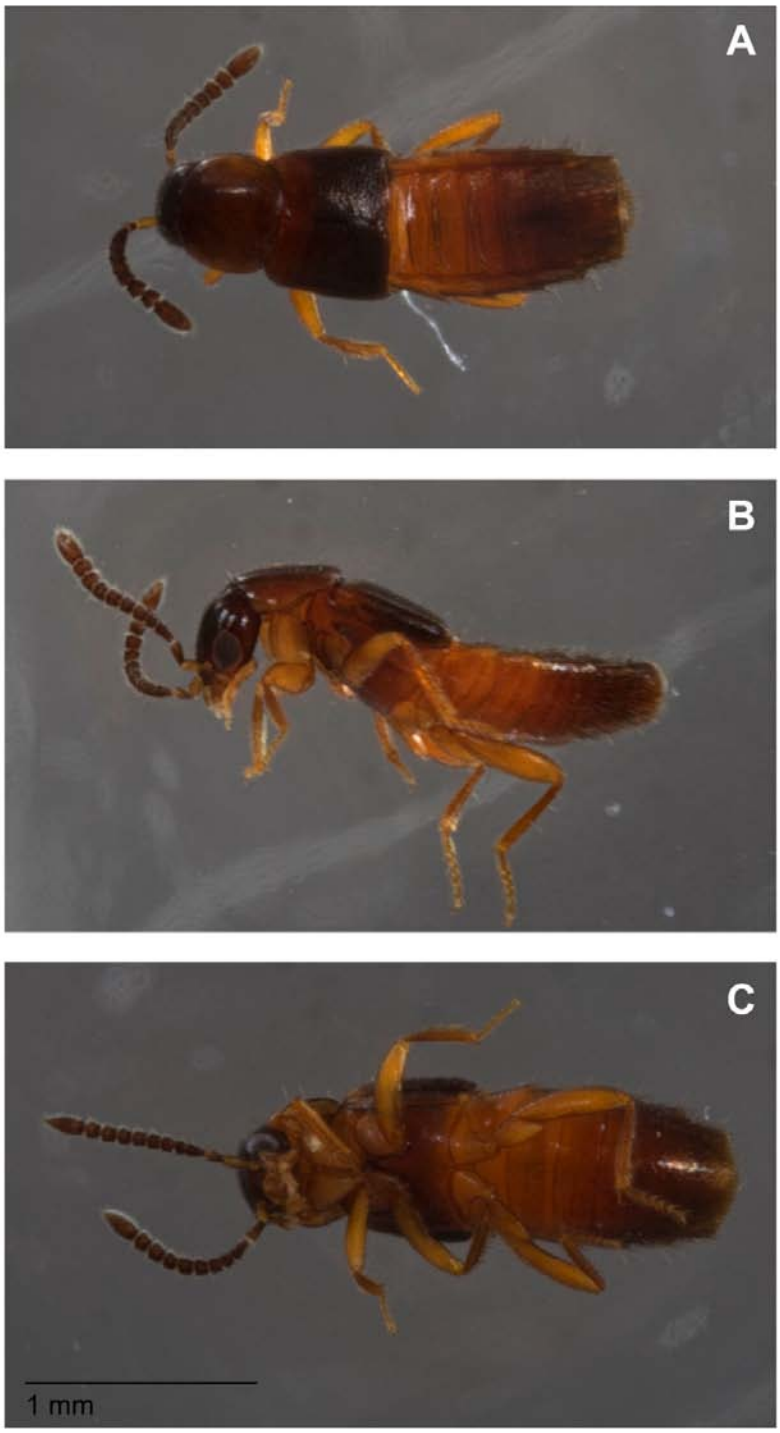
Supplementary Figure S4-5. Non-assayed species of millipede (Diplopoda: Polydesmida) found within bivouac B of *Labidus praedator* in .**A** dorsal, **B.** lateral, and **C** ventral view; only one individual of this species was encountered



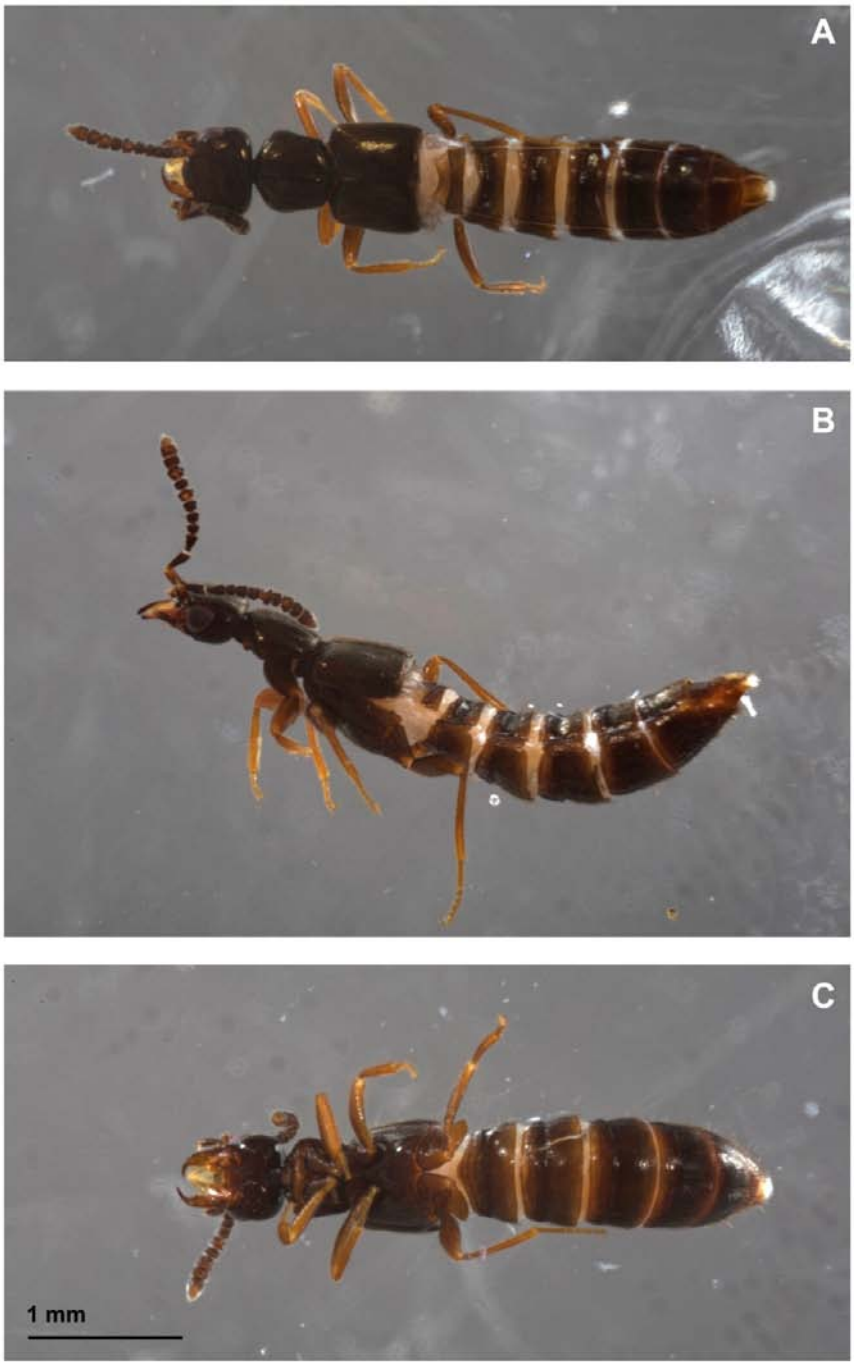
Supplementary Figure S4-6. Two Acari species found within Bivouac B in dorsal (A&D) lateral (B&E) and ventral (C&F) view; A-C correspond to Acari sp. 1, D-F correspond to Acari sp. 2

Supplementary Figure S4-7. Inquiline beetle (Staphylinidae: Scydmaeninae) encountered within bivouac B of *Labidus praedator* in lateral view (A) and ventral view (B); only one individual encountered

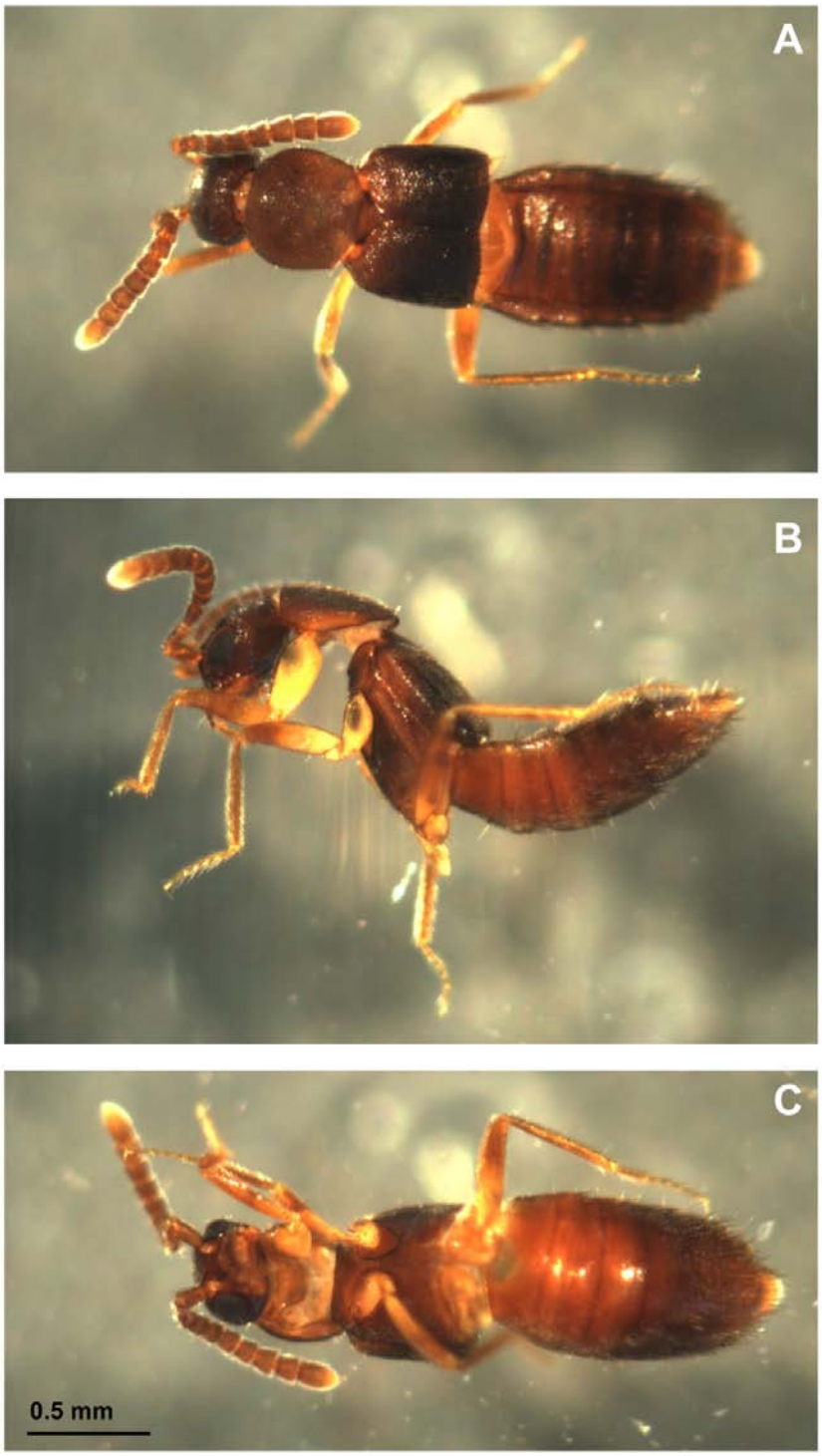
Supplementary Figure S4-8. Staphylinidae sp. 1 encountered within bivouac B of *Labidus praedator* in dorsal view (A), lateral view (B), and ventral view (C)



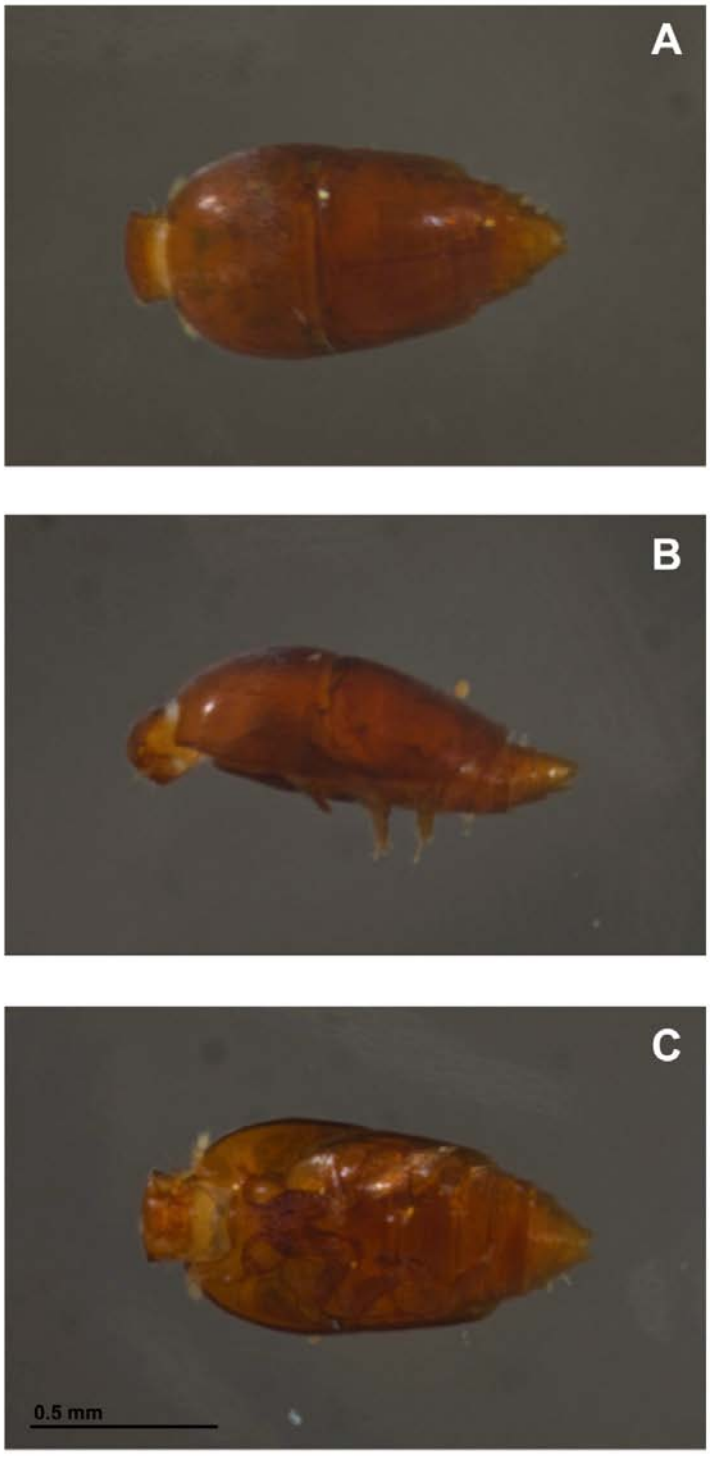
Supplementary Figure S4-9. Staphylinidae sp. 2 encountered within bivouac B of *Labidus praedator* in dorsal view (A), lateral view (B), and ventral view (C)



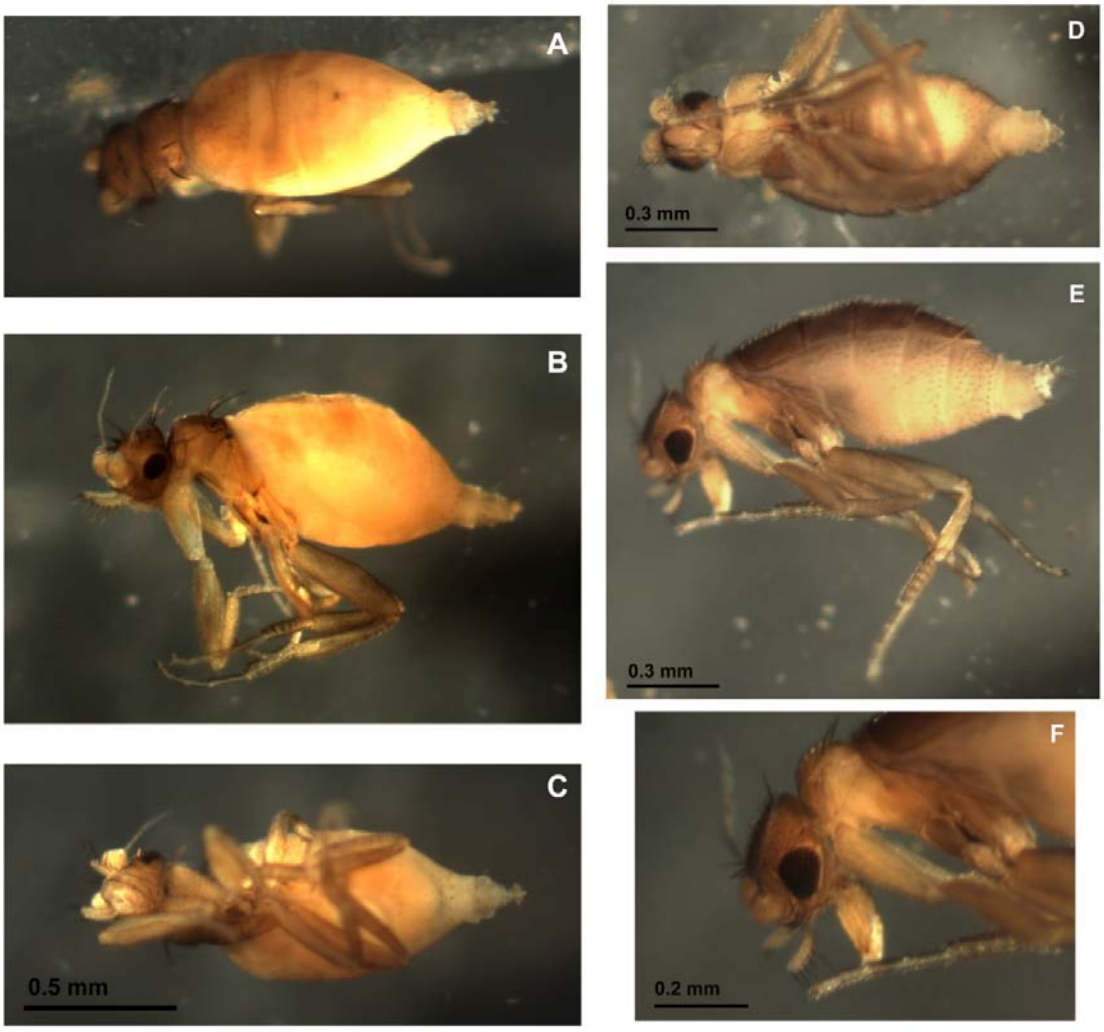
Supplementary Figure S4-10. Rove beetle Staphylinidae sp. 3 encountered within bivouac B of *Labidus praedator* in dorsal view (A), lateral view (B), and ventral view (C)



Supplementary Figure S4-11. Rove beetle Staphylinidae sp. 4 encountered within bivouac B of *Labidus praedator* in dorsal view (A), lateral view (B), and ventral view (C)

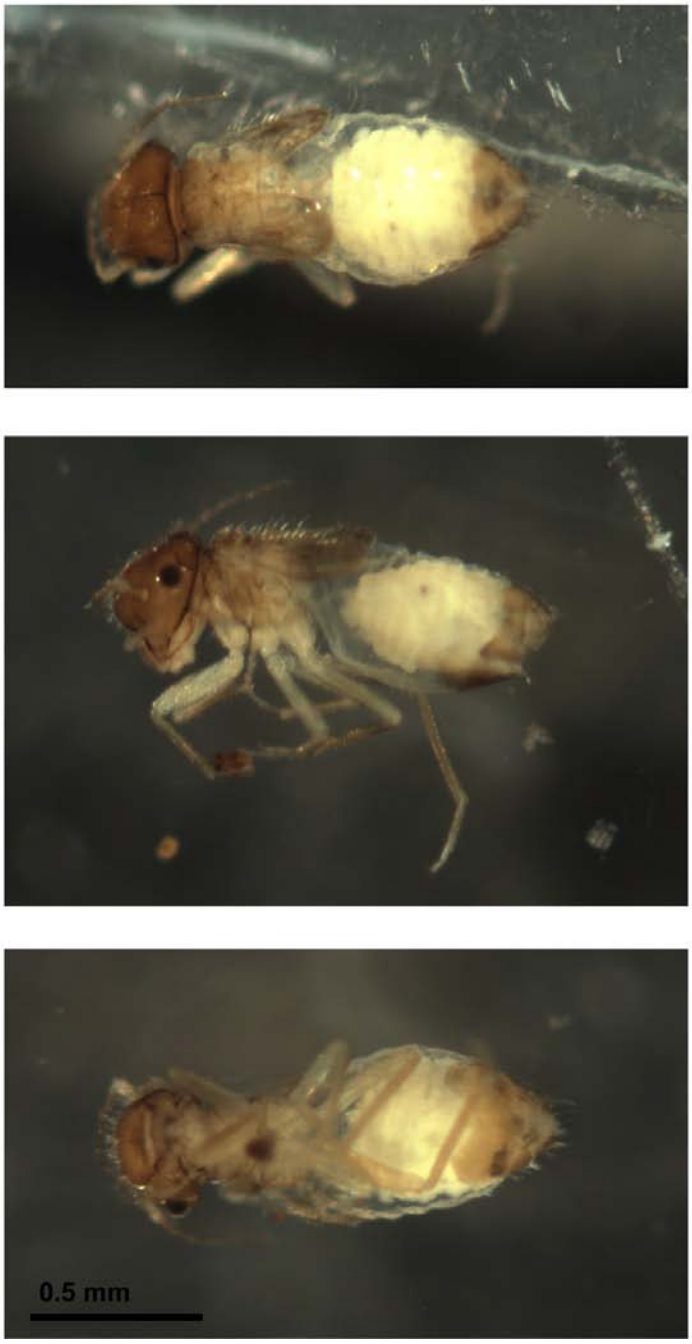


Supplementary Figure S4-12. Inquiline beetle (Subfamily: Cephaloplectinae) encountered within bivouac B of *Labidus praedator* in dorsal view (A), lateral view (B), and ventral view (C)



Supplementary Figure S4-13. Two morpho-species of wingless phorid encountered in the surface dump material of *Labidus praedator* bivouac B; Phoridae sp. 1in images A, B & C; Phoridae sp. 2 in images D, E & F

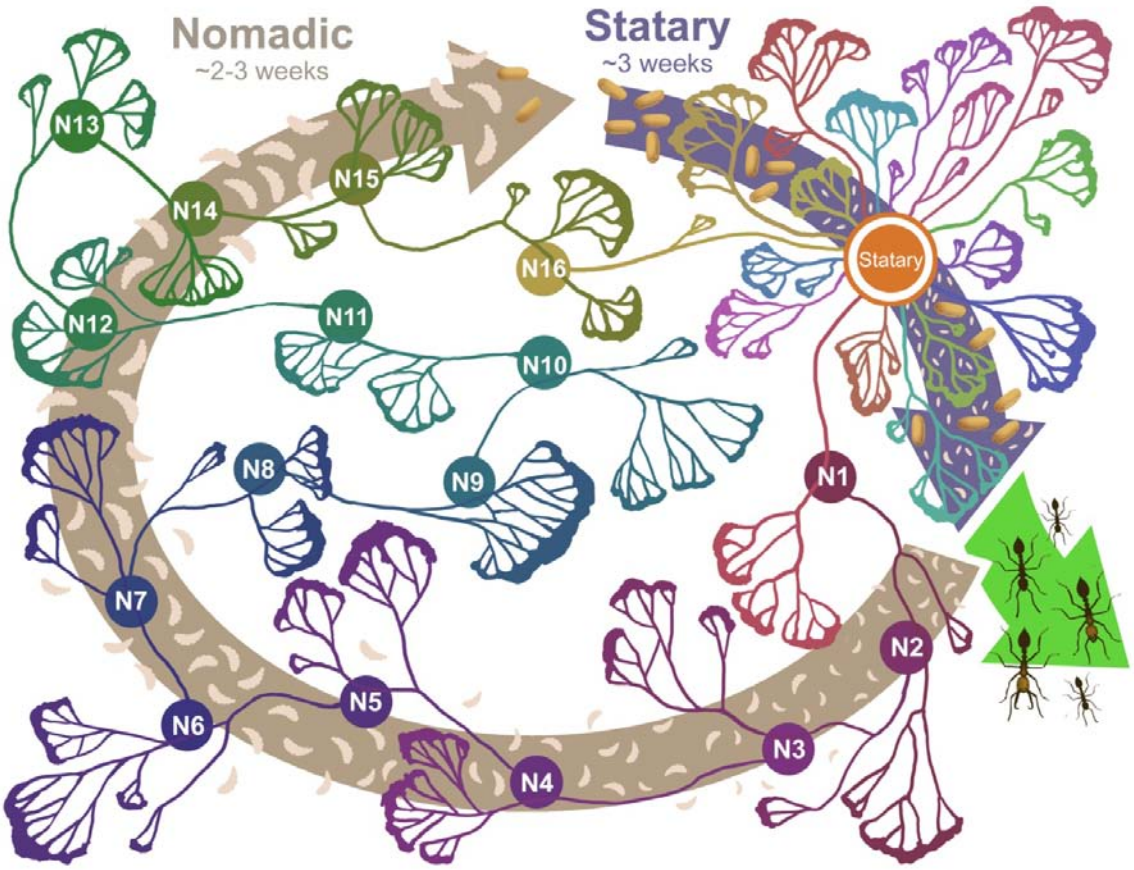
Supplementary Figure S4-14. Phoridae sp. 3; several hundred individuals arrived at bivouac of *Labidus praedator* when high ant-density portions of it were excavated



Supplementary Figure S4-15. A possibly incidental Psocopteran recovered from surface dump samples of bivouac B

Supplementary Figure S4-16. Collembola sp. (Entomobryidae) collected on surface dump materials of intact bivouac B of *Labidus praedator*; one other species (Poduroomorpha) observed but not collected

APENDIX C: Supplementary material for chapter 5



Supplementary figure S5-1. Nomadic and statary phases of *Eciton burchellii* life cycle depicting brood age and typical colony raid and emigration patterns, based on previous description (Schneirla 1938; Schneirla, Brown & Brown 1954; Rettenmeyer 1963)

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Baudier KM, Kaschock-Marenda SD, Patel N, Diangelus KL, O'Donnell S, Marenda DR. (2014) Erythritol, a non-nutritive sugar alcohol sweetener and the main component of Truvia®, is a palatable ingested insecticide. *PLoS ONE,* 9: e98949.

Invited talks:

Baudier KM. Social thermoregulation along elevational clines: lessons from a Neotropical army ant. Philadelphia Evolution Group. 10 October 2016 Philadelphia, PA

Baudier KM. La tolerancia térmica y la termorregulación de un grupo de hormigas legionarias neotropicales. Reserva Biológica del Bosque Nuboso de Monteverde. 25 April 2016. Monteverde, Puntarenas, Costa Rica.

Baudier KM. Thermal tolerance in Neotropical army ants: body size, microhabitat & elevational effects. American Entomological Society Monthly Meeting. 26 March 2014. Academy of Natural Sciences, Philadelphia, PA.